FINAL REPORT

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Project Title:

Addressing crucial American Eel life history questions: Baseline data on the age and sex composition of American Eels in South Carolina, with a comparison of different ageing methodologies

Background:

American Eel (*Anguilla rostrata*) are of concern both nationally and locally, being twice petitioned (most recently in 2010) for listing as "threatened" or "endangered" under the Endangered Species Act (ESA) by the U.S. Fish and Wildlife Service and listed as a species of highest priority by the South Carolina State Wildlife Action Plan (SWAP). For both national petitions, 12-month findings (most recent in October 2015) resulted in a negative outcome (no ESA listing), though they consider American Eel a "species of concern" and evidence suggests the stock is severely depleted compared to historic levels. Along the U.S. Atlantic coast, the Atlantic States Marine Fisheries Commission (ASMFC) manages American Eel as one panmictic stock, due to mixing of early life stages (eggs, pre-larval, and leptocephali) in the Sargasso Sea spawning grounds. A recent benchmark stock assessment concluded the entire stock is depleted compared to historic levels (ASMFC 2012), with a 2017 update suggesting the stock remains depleted in U.S. waters with abundance trends suggesting stable to downward trending abundances (ASMFC 2017); a new benchmark stock assessment is currently underway through the ASMFC assessment process. The 2017 update assessment suggested American Eel are at or near historically low levels due to a combination of historical overfishing, habitat loss, food web alterations, predation, turbine mortality, environmental changes, toxins/contaminants, and disease (ASMFC 2017). Internationally, the International Union for the Conservation of Nature (IUCN) listed American eel as endangered on the IUCN Red List in 2014, due to primarily meeting their criteria of an "observed, estimated, inferred or suspected population size reduction of ≥10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased or may not be understood or may not be reversible" (Jacoby et al. 2014).

Critical to our concerns for the American Eel population in the state of South Carolina are the findings of the 2017 assessment report (ASMFC 2017). The only young of the year (YOY) index from South Carolina, a YOY index based on fyke net catches in Goose Creek Reservoir, suggested a significant downward trend in relative abundance (ASMFC 2017). Similarly, the only index of the yellow eel life stage in South Carolina – a statewide upper estuary electrofishing survey – suggested a downward trend in the abundance of yellow eels across the state (ASMFC 2017). When combined with other relative abundance data from Florida, Georgia, and North Carolina, there was a clear decline in YOY and yellow eel stage American Eels across the four states since 2001 (ASMFC 2017). These results suggested at the meta-population scale, there is even greater concern for the status of American Eel in the state of South Carolina and an increasing probability the population is approaching imperiled status. Subsequent to the assessment, the declining trends have not reversed, with 2019 and 2020 being record low yellow eel abundance years across the state, hinting at a steepening of the decline (Figure 1; J. Ballenger, pers. comm.).

Unfortunately, major impediments exist to our complete understanding of the drivers of population change for American Eel throughout their range, which contributes to our incomplete understanding and elevated levels of uncertainty about population status coastwide and at the meta-population scale from assessments. As such, the 2017 update assessment listed several critical research priorities, though the only recommendation that reviewers indicated must be addressed prior to a new benchmark stock assessment was to "conduct intensive age and growth studies at regional index sites to support development of reference points and estimates of exploitation" (ASMFC 2017). The 2015 SC SWAP also identified the need to "determine size, sex, and age structures for each sub-population of American Eels" as a conservation recommendation (SWAP 2015). We need region-specific life history studies of American Eels because they are noted for having extremely plastic life histories, exhibiting large differences in growth and reproductive traits throughout their range (Hansen and Eversole 1984; Helfman et al. 1984), though no such information exists for American Eels found in South Carolina and what information is available primarily derives from mid-Atlantic and northeastern watersheds (SWAP 2015). Where studied, researchers have postulated numerous environmental drivers for explaining the high degree of plasticity, including habitat quality, American Eel density and parasite (*Anguillicoloides crassus*) infection. To date, the only known study aimed at addressing this research priority is the collection of routine age information from American Eels found in the Chesapeake Bay via the Maryland Department of Natural Resources (ASMFC 2017); researchers have not initiated any dedicated research devoted to understanding age and growth of American Eel in the states of FL, GA, SC, or NC to our knowledge.

Also included as research recommendations in the 2017 update stock assessment of American Eel was a need to improve our understanding of:

- the distribution and frequency of occurrence of American Eels along the Atlantic Coast over time, with a recommendation being to collect biological information by life stage including length, weight, age, and sex of eels caught in fishery-independent sampling programs;

- the impact of *Anguillicoloides crassus* on American Eel, with research needed to understand the effect of the swim bladder parasite on the American Eel's growth and maturation which would aid our understanding of the impact of the introduction of *A. crassus* into areas presently free of the parasite;

- spawning and maturation, including identifying triggering mechanisms for metamorphosis to mature adult, silver eel life stages, with specific emphasis on the size and age of the onset of maturity, by sex, and specific acknowledgement that a maturity schedule (by size and age) would be extremely useful in combination with migration rates; and

- habitat needs and availability, with a need to assess characteristics and distribution of American Eel habitat and value of habitat with respect to growth and sex determination (ASMFC 2017).

Arnott & de Buron (2015) undertook preliminary work to address these needs in South Carolina, though they processed a limited of number of samples (n = 230 histological samples; n = 300 age samples) and limitations of these data are clear when partitioning the data by sex, size, age, time of year, and location. Further, they processed a small number of samples from silvering individuals and therefore they could not address questions concerning the onset of maturity. Additionally, in some European populations, research suggests infection by the parasitic nematode *A. crassus* triggers early silvering (Lefebvre et al. 2008). Here in South Carolina, SWAP documents *A. crassus* as an invasive species of concern (SWAP). Earlier work done by the South Carolina Department of Natural Resources (SCDNR) Inshore Fisheries section has documented the seasonality of *A. crassus* infection and its relationship to habitat type and eel total length (Hein et al. 2014). However, we still do not understand the relationship between *A. crassus* infection and sexual maturity and other life history parameters.

To better understand patterns of life history, we need an accurate and consistent method for aging. In the most recent benchmark stock assessment, the ASMFC also cited a need for standardization of aging methods (ASMFC 2012). Currently, there is an ongoing collaborative eel aging effort between many Atlantic states' researchers supported by the ASMFC. The results of a preliminary exchange of otoliths between states resulted in a lack of agreement in both preparation of otoliths for aging purposes and the ultimate assignment of annulus counts. This led to an in-person workshop in January 2017 where prepared otoliths were read and discussed by the group, resulting in a deeper understanding of each state's current methods and increased confidence by the group in arriving at agreement when counting annuli. That said, substantial lack of agreement in the preferred preparation method (mounting and polishing a section versus whole mounted otoliths) remained, with preparation method contributing to differences in age determination. They recommended an in-depth analysis of each method, with a primary goal being the construction of translation tables for ages between the two methodologies.

To these points, the overall goal of this project was to leverage currently collected histological and otolith samples to address critical data gaps identified in recent American Eel stock assessments.

1. Process SCDNR archived histological samples from American Eels

2. Process SCDNR archived otolith samples via two separate techniques recommended by the recent ASMFC ageing workshop conducted for American Eels

3. Assess the precision of age determinations developed using each processing method via inspection of bias plots and statistical methodologies, including (but not limited to) the use of average percent error and symmetry tests. We will assess differences in the precision of the independent ageing methodologies using paired t-tests and develop translation tables, if possible, to facilitate conversion of age estimates derived using one technique to the other.

4. Assess the age structure of American Eels in South Carolina

5. Assess the sex ratio, size-at-maturity, and age-at-maturity of American Eels in South Carolina

6. Use multi-variate analysis to identify influential covariates influencing the apparent life history (e.g., growth, sex ratio, size-at-maturity, age-at-maturity) of eels in South Carolina. Potential covariates considered may include year or time of capture, location of capture, environmental conditions, and *A. crassus* infection.

Objectives:

The overall goal of this project was to leverage currently collected histological and otolith samples to address critical data gaps identified in recent American Eel stock assessments

Job 1:

Process SCDNR archived histological samples from American Eels

Accomplishments:

From 2012-2018 we opportunistically took gonad histological samples from American Eels sacrificed from SCDNR fishery-independent and fishery-dependent collection programs, with most samples deriving from our fishery-independent electrofishing survey of low salinity (< 8 PSU), tidally influenced, upper estuary habitats. Regardless of source, SCDNR staff measured all deceased American Eels to the nearest millimeter total length and brought individuals back to the lab for a complete workup, including the collection and formalin fixation of macroscopically identified gonadal tissues. Using standard hematoxylin-eosin staining techniques of paraffin-embedded gonads, we prepared histological slides of confirmed gonad tissues (n = 1,141; 93.4% of proposed samples) and then microscopically assessed the sex of all individuals. These eels came from the low salinity, tidally influenced portions of the lower Combahee River (n = 146), Edisto River (n = 93), Ashley River (n = 185), Cooper River (n = 398), and Winyah Bay (n = 205) sampled by the electrofishing survey and some additional in-river sampling of American Eels in the Savannah River (n = 8), Santee River (n = 27), Congaree River (n = 1), Great Pee Dee River (n = 25), and Little Pee Dee River (n = 20). An additional 33 American Eels were collected from unknown locations across South Carolina.

Two independent readers determined the sex and maturity stage of all gonad histological samples from American Eels, following the reproductive stage terminology presented in Brown-Peterson et al. (2011). An additional subgrouping of female maturity stage recording early-development and mid-development were recorded. We classified early-development as the presence of cortical alveolar and oil droplet structures; mid-development was categorized by the presence of vitellogenic 1 and 2 stage oocytes (vtg1 and vtg2, respectively). No stages beyond vtg1 or vtg2 were seen in any female reproductive tissues. This is not unexpected given American Eel's semelparous life history and suspected location of spawning in the Sargasso sea.

Sex was undifferentiated in 232 of the eels (the gonads were either too small to isolate, or sexual features were indistinguishable when we examined histologically prepared gonad samples under a microscope). Undifferentiated eels were observed up to 525 mm TL with the presence of up to 10 annuli (12 years of age). These, however, are outliers and 95% of the samples were differentiated by 325 mm and 8 years (6 annuli) of age. Among the remaining eels, there were 163 males, and 746 females. The proportion of males and females varied significantly with significant relationships seen with sex as the response variable, and eel total length and time of year as covariates ($p<0.05$). Generally, males were smaller than females, and were primarily seen from May-December (83.6%, Figure 2) In females, early development was present all year round while mid-development, or presence of vtg 1 and vtg 2, was primarily observed in the fourth quarter (September-December, Figure 2). The relationship between mid-development maturity stage to total length (mm) and month of capture was evaluated with female maturity stage as a binary response (early vs. mid-development), and total length and month of capture as the covariates ($p <0.05$).

Significant deviations:
Gonadal materials were not successfully retained from 70 individuals of the proposed 1,211, primarily due to non-sampling of reproductive tissues during initial lab workup.

Job 2

Process SCDNR archived otolith samples via two separate techniques recommended by the recent ASMFC ageing workshop conducted for American Eels

Accomplishments:
In addition to the collection of gonadal tissues, we collected otoliths from American Eels sacrificed for laboratory life history work-up. We were successful in collecting otoliths from 1,106 individuals, of which both otoliths were obtained from 1,081. Otoliths removed in the lab were placed in 100% ethanol for

two weeks and then cleaned and stored dry until processed. The primary otolith processing methodology employed by SCDNR staff is an "otolith thin section" technique whereby an otolith is embedded in epoxy resin and then a transverse cross section of approximately 0.5mm thickness is taken through the core of the otolith using an IsoMet low speed saw with diamond wafering blades. This section is mounted on a glass slide using CrystalBond thermoplastic adhesive and then ground to a desired thickness of around 0.2-0.3mm using Buehler grinding pads (400-1200 grit) and polished with Buehler 0.3 micron polishing compound (ASMFC 2017b). Hereafter, this technique will be referred to as the "OTS method". Samples from all 1,106 individual American eels were processed in this manner. For the 1,081 specimens for which two sagittal otoliths were available, we processed the second otolith using a "whole otolith preparation" technique followed by Maryland Department of Natural Resources (ASMFC 2017b). Whole otoliths were placed convex side up on glass microscope slides and mounted with CrystalBond. Annulus counts were assigned under the microscope (as below) in this fashion if possible, otherwise the dorsal surface of the otolith was ground slightly until readable, then polished and covered in CrystalBond if necessary. Hereafter, this technique will be referred to as the "WO method". Regardless of processing technique, staff counted annuli at 10-75x magnification on a Nikon SMZ-U dissecting microscope by viewing through the objectives and/or the projected image on a computer monitor using an Infinity1 microscope camera. Both transmitted and reflected light were used to increase confidence in annulus count. Each sample (1-2 per fish) was read by two independent age readers. Previous marginal increment analysis had confirmed the formation of a single annulus per year (Arnott and de Buron 2015; ASMFC 2018).

Each prepared sample (OTS and WO method) was read by two independent age readers. Readers determined the annulus count of each sample in a double-blind fashion, with no knowledge of fish capture date or fish size. This minimizes bias of interpretation resulting from such knowledge when determining age using hard structures in fish species. Individual readers made no attempt to evaluate edge type or assign a quality code as is common for many other species. The difficulty of interpretation and structure size made such determinations arbitrary. No reader was considered superior to the other with regards to ageing of American Eel, so they were arbitrarily assigned as Reader 1 and Reader 2. If independent annulus counts differed between readers, a consensus read was conducted to develop a consensus annulus count; if a consensus was not reached, the sample was removed from all consensus annuli count-based analyses.

Significant deviations:
Instead of 1,221 age estimates being available for investigation, a total of 1,106 thin-sectioned otoliths and 1,081 whole otoliths were processed in part due to difficulties processing the small, fragile otoliths as well as errors in archiving the samples.

Job 3

Assess the precision of age determinations developed using each processing method

Accomplishments:
Aging bias estimates between readers within a method and between consensus ages using the OTS and WO methods were calculated using the *FSA* package (Ogle et al. 2021) in R (R Core team 2020). We used annuli counts as a proxy for age for all comparisons. For each comparison, we evaluated potential for bias using three symmetry tests: Bowker Test (Hoenig et al. 1995), Evans-Hoenig Test (Evans and Hoenig 1998), and McNemar Test (Evans and Hoenig 1998), then computed measures of precision (percent absolute agreement, average coefficient of variation (ACV), and average percent error (APE)), and developed a Bland-Altman bias plots.

Otolith Thin-Section (OTS) Method
For ages determined using the OTS method, the results of the symmetry test indicated no pronounced bias in annulus counts between readers (Table 1), suggestive of a high degree of precision and reproducibility of age estimates using this method. Other measures of precision corroborate these results. There was an absolute agreement between readers of 69% (Table 1), with most of the disagreements within one annulus count. Percent agreement to within one annulus was 94.3%, though absolute annulus count differences between readers ranged up to 5 annuli. Finally, the ACV and APE between readers was 8.0% and 5.7%, respectively (Table 1). While slightly higher than the suggested 5% ACV recommended by Campana et al. (2001), these data are precise given the difficulty in ageing American Eels.

Bland-Altman plots suggested different ages of bias depending on the reader assigned as the reference age. When Reader 1 was assigned as the reference age, the Bland-Altman plot suggested bias between readers for American Eels possessing 3, 6, 7, and 9 annuli, though the degree of bias was small for all reference annuli counts (Figure 3). When Reader 2 was assigned as the reference age, the Bland-Altman plot suggested bias between readers for American Eels possessing 0 or 1 annulus, though once again the degree of bias was small for all reference annuli counts (Figure 4). These results corroborate the results of the symmetry tests, which showed no overall significant bias between reads using the OTS method (Table 1).

Whole Otolith (WO) Method
For ages determined using the WO method, the results of the symmetry test indicated pronounced bias in annulus counts between readers (Table 1), suggestive of a lack of precision and inability to reliably reproduce annulus counts by different readers. Other measures of precision corroborate these results. There was an absolute agreement between readers of 43% (Table 1). However, like the case with the OTS method, most of the disagreements were within one annulus count. As such, percent agreement to within one annulus was 79.8%, or 15% lower than observed using the OTS method. Finally, the ACV and APE further indicated the WO method was less precise, with an ACV and APE of 25% and 18%, respectively (Table 1). These estimates were much higher than the suggested 5% ACV recommended by Campana et al. (2001), suggesting the WO method for American Eel produces imprecise estimates of age.

Bland-Altman plots suggested different ages of bias depending on the reader assigned as the reference age. When Reader 1 was assigned as the reference age, the Bland-Altman plot suggested bias between readers for American Eels possessing 0 and 3-9 annuli, with a clear pattern of increasing bias with annulus count (Figure 5). The direction of the bias indicated Reader 2 tended to underage with respect to Reader 1. When Reader 2 was assigned as the reference age, the Bland-Altman plot continued to suggest significant bias between readers for American Eels, with most well represented groups in the plot indicating bias between readers (Figure 6). These results corroborate the results of the symmetry tests, which showed significant bias between readers using the WO method (Table 1).

Comparison of Processing and Ageing Methods
For a final comparison of the precision between the two independent otolith processing and ageing methods, we used consensus annulus determinations. These consensus annulus determinations represent the best estimate of annulus counts, as they result from consultations between the two readers on any disagreements with their independent reads. Such consensus annulus counts represent the best science available to stock assessments, and as such would be the information provided for

stock assessment purposes. As detailed above, previous results suggested the OTS method provided more precise estimates, so consensus ages based on this method were used as the reference age.

Results of symmetry tests indicated pronounced bias in annulus counts between methods (Table 1), suggesting age estimates made by the two independent methods are not comparable. Other measures of precision corroborate this conclusion, with an absolute agreement in consensus ages between methods of 44% (Table 1). Further, the ACV and APAE indicated lack of agreement of between methods, with an ACV and APE of 20% and 14%, respectively (Table 1). These estimates were much higher than the suggested 5% ACV recommended by Campana et al. (2001). Finally, the Bland-Altman plot suggests the differences in annulus counts between the two methods is due to under-ageing of American Eels via the WO method, with the degree of under-ageing becoming more pronounced at older ages (Figure 7).

Annulus Count Translation Table
The above results highlight the superiority of the OTS processing and ageing method for American Eels for the generation of annulus counts. Between-reader comparisons suggest the OTS method provides for more precise and reproducible annulus counts (Table 1 and Figures 3-6). The comparison of consensus ages developed using the two independent methods highlights the bias, relative to the preferred ageing methodology, introduced by use of the WO method (Table 1). The WO method results in significant under-ageing of American Eels relative to the reference methodology, with the degree of under counting of annuli increasing with age (Figure 7).

For these reasons, we developed an ageing method translation table to facilitate the conversion of WO method-derived annulus counts to OTS method-derived annulus counts (Table 2). Such an approach will be necessary to create a standard “age currency” for stock assessment purposes. Failure to account for differences in ageing methodologies could result in spurious conclusions with regards to changes in age structure and stock trajectories.

<u>Significant Deviations</u>
None

Job 4

Assess important life history parameters: Assess the age structure, sex ratio, size-at-maturity, and age-at-maturity of American Eels in South Carolina

<u>Accomplishments:</u>
Since 2010 we have collected detailed life history information from 1,613 individual American Eels through our fishery-independent sampling programs, most commonly through electrofishing surveys (n = 1,569). Herein, we summarize life history information, as gleaned from these samples, to provide a more robust picture of the life history of American Eels in South Carolina. We collected these eels throughout the coastal plain region of South Carolina, with most individuals captured in upper estuary, tidally influenced, low salinity habitats routinely sampled by a long-term electrofishing survey (Figure 8). Recorded biological parameters for sampled eels include total length (mm), weight (g), life stage (yellow eel or silver eel), degree of swim bladder impairment due to *A. crassus* infection (none, low, moderate, high), the presence and number of adult *A. crassus* in the swim bladder at time of collection, sex (both macroscopically and histologically), maturity stage (immature vs mature), stage of female development in developing stage individuals (early- or mid-development), maximum oocyte diameter, annuli count by ageing method, and age by ageing method (Table 3). Note age can be calculated as annuli count + 2, as recommended by previous research (Arnott et al. 2015), however age-based estimates were performed

based on annulus count in the current report. Further, based on results of Job 3, all age-based analyses use OTS method derived annulus counts.

Size structure:
American Eels retained for life history analysis ranged in size from 58 to 900 mm TL, with a mean ± 95% CI size of 382 ± 6.56 mm TL; the median size for eels was 365 mm TL. The overall length frequency of eels collected since 2010 was bimodal in nature, with a peak between 280-340 mm TL and a second peak between 460-520 mm TL (Figure 9). Overall size range of eels retained by quarter remained constant, though there was a propensity to see fewer small eels as time progressed throughout the year; maximum size was nearly identical in all four quarters (Figure 10 and Figure 11). Across years, while the number of samples varied, in general the size of American Eels retained remained constant, outside of 2015 when there were more small American Eels available (Figure 12 and Figure 13). Finally, no clear spatial pattern emerged concerning length frequency by location – the range of sizes encountered, nor frequency of certain sizes did not appear to vary clearly as a function of space (Figure 14 and Figure 15).

Age structure:
American Eels retained for life history analysis exhibited annulus counts ranging from 0 to 13, with a mean ± 95% CI annulus count of 4.1 ± 0.098; the median annulus count for eels was four. If converted to age using the annuli count + 2 method posited by Arnott et al. (2015), ages of American Eels encountered by the survey ranged from 2 to 15 years old. The age structure of American Eels collected since 2010 in South Carolina shows a clear peak, with eels possessing 3-5 annuli being most commonly encountered (Figure 16). The annulus count composition of American Eels did not vary substantially by quarter, though there was a tendency for older fish (those possessing greater annulus counts) being relatively more common from July-December (Figure 17 and Figure 18). Similarly, there was little indication that annulus count composition of American Eels encountered in South Carolina changed substantially from year-to-year, with limited to no signal of particularly strong year classes over the years available (Figure 19 and Figure 20). Finally, we saw no clear spatial pattern concerning annulus count composition (Figure 21 and Figure 22).

Size-at-age:
<u>Age-Length Key</u>
Given the annual sample sizes, sample sizes by sex, and general lack of differences in sizes or ages of American Eels encountered within a year or spatially, we pooled all available total length and annulus count information for the development of pooled age-length keys. When the available data were subset to those specimens for which both a total length and OTS method-derived annulus count were available (n = 1,374), total lengths and annulus counts ranged from 84-900 mm TL and 0-13 annuli, respectively. Age-length keys were developed after binning raw total lengths to 20 mm bins, with individual bins ranging from 80-99 mm TL to 900-919 mm TL.

An initial raw age-length key using available data was developed (Table 4), which generally showed an increasing probability of more annuli with total length (Figure 23). Sample size was an issue with some individual bins, with less than five individual fish being aged for bins with a minimum length less than 120 mm TL and greater than 660 mm TL, as well as no specimens between 800-899 mm TL (Table 4).

Such a situation of sparse age-length keys is common in fisheries, particularly at the smallest and largest size classes encountered. To partially account for the sparse nature of some of the data, we also developed smoothed age-length keys using multinomial regression (Gerritsen et al. 2006; Ogle 2018).

These analyses were performed using the *nnet* (Venables and Ripley 2002) package available in R (R Core Team 2020). The resulting smoothed age-length key can be found in Table 5 and Figure 24. This age-length key accounts for the sparse data observed in smaller and larger size classes of American Eel. Note, as was observed in the raw data, the smoothed age-length key still suggests much variability in size-at-age for South Carolina captured American Eels (Figure 24).

von Bertalanffy Growth Curve

Using the same data set available for the development of age-length keys, we also evaluated the size-at-age and growth of American Eels in South Carolina. Growth was modeled using the von Bertalanffy growth function,

$$L_t = L_\infty * \left(1 - e^{-k(t-t_0)}\right),$$

where L_∞, k, and t_0 are the three parameters to be estimated via non-linear regression and represent the asymptotic total length (mm), Brody growth coefficient, and theoretical age when size would by 0, respectively. L_t and t represents the observed total length (mm) and annulus count based on OTS method ageing protocols, respectively. Confidence intervals about parameter estimates and predicted mean size-at-age were estimated via 10,000 bootstraps of the raw data.

When pooled by sex, von Bertalanffy growth function parameter estimates for L_∞, k, and t_0 were 619 mm TL, 0.19 year^{-1}, and -1.3 years, respectively (Table 6 and Figure 25). The resultant predicted mean size-at-age, relative to the raw observed data, is provided in Figure 26. While there is variability in raw size-at-age, there is a relative rapid increase in mean size-at-age of American Eels in South Carolina before approaching near asymptotic levels by the time 10 annuli are formed. Figure 27 shows the stark contrast a von Bertalanffy growth curve would produce using WO method-derived annulus counts relative to the OTS method-derived curve. Use of the WO method growth curve would result in an overestimate of size-at-age, resulting in inaccurate biomass estimates in stock assessments.

We also developed von Bertalanffy growth curves by sex, owing to the apparent clear sexual differentiation in growth patterns (Figure 26). To develop sex-specific growth curves, we included all undifferentiated American Eels for both sexes. This implicitly assumes differences in growth patterns between the sexes emerge once sexual differentiation occurs, which is commonly observed in other species. Male von Bertalanffy growth curve parameters (Table 6 and Figure 28) and predicted size-at-age suggest much slower growth than observed in females (Table 6, Figure 29 and Figure 30). The difference in size-at-age is evident by the time one annulus is formed and becomes more pronounced with subsequent annulus formations (Figure 30).

Size-at-maturity:

We used logistic regression models with binary immature/mature response data to assess relationships between maturity and eel total length, with maturity defined using the terminology posited by Brown-Peterson et al. (i.e., early- and mid-developmental stages were present in our data; 2011). Logistic regression models were fitted separately to the male and female maturity data (Table 7); confidence intervals about parameter estimates and maturity ogives were developed using 10,000 bootstraps of original data.

For females, season (Jan-Mar, Apr-Jun, Jul-Sept, Oct-Dec) was not significant. However, maturity increased significantly with total length ($p < 0.0001$), with 50% maturity at 450.3 mm TL (95% CI: 442.5-457.7 mm TL; Figure 31 and Table 8). For males, total length and maturity were not significantly associated with one another (Figure 32). This is due to males' narrow size range in the available data and the high degree of overlap in maturity stage across the size range. This resulted in an estimated size-at-50% maturity for males of 248.5 mm TL though the 95% CI of this estimate ranged from -144.6 to 497.3 mm TL (Table 8 and Figure 32) Seasonality was significantly associated with maturity for males, likely relating to the lack of males present in our samples from Jan-Apr.

Age-at-maturity:
We used logistic regression models to analyze age at maturity with annulus count as proxy for age. True age can be estimated as annulus count +2, based on previous recommendations by Arnott et al, 2015. Logistic regression models were fitted separately to the male and female maturity data (Table 7). Confidence intervals about parameter estimates and maturity ogives were developed using 10,000 bootstraps of original data.

For females, season (Jan-Mar, Apr-Jun, Jul-Sept, Oct-Dec) was not significant. However, maturity increased significantly with annulus count ($p < 0.0001$), with 50% maturity at 4.2 annuli (95% CI: 4.0-4.5 annuli; Table 8 and Figure 33) For males, annulus count at 50% maturity was 2.3 annuli (955 CI: -0.07-3.4 annuli; Table 8 and Figure 34). Like size-at-maturity, the maturity ogive for males was not estimated precisely. Again, seasonality was significantly associated with maturity for males ($p < 0.0001$), likely relating to the lack of males present in our samples from Jan-Apr.

Significant deviations
None

Job 5
Use multi-variate analysis to identify influential covariates influencing the apparent life history (e.g., growth, sex ratio, size-at-maturity, age-at-maturity) of eels in South Carolina.

Accomplishments
We used multiple logistic regression to evaluate the influences that location, season, and presence of *A. crassus* adults had on size- and age-at-maturity of female and male American Eels. All analyses were performed on male and female data separately. While the primary variable of interest was how probability of maturity varied as either a function of size (total length, in mm) or annulus count, the inclusion of additional covariates allowed us to investigate how this underlying relationship was modified by each covariate. In all cases, a full model was developed including the primary effect of interest (total length or annulus count for size-at-maturity and age-at-maturity analyses, respectively) and discrete covariates representing location (from five areas, South to North): Combahee River, Edisto River, Ashley River, Cooper River, and Winyah Bay), season (four seasons: Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec) and presence of *A. crassus* adults in the swim bladder at time of dissection (0 = not present; 1 = present). From the full model fit, backwards selection using AIC was used to identify the best fit model, dropping covariates as needed. No interaction effects were included in any model.

Size-at-maturity
Female
The best fit multiple regression model for female size-at-maturity dropped the covariate season from the final model. All other covariates (total length, presence of adult *A. crassus* in swim bladder, and river system) were retained. Though location was retained in the best fit model, there was no clear discernible trend with regards to size-at-maturity of female American Eels with latitude, as we may have expected (Figure 35); the results indicated American Eel females tended to mature slightly earlier in the

Winyah Bay (northernmost stratum) and Combahee River (southernmost stratum) strata (Figure 35). The effect of the presence of adult *A. crassus* in the swim bladder was clearer, leading to earlier maturity of female American Eels when infected by the parasite (Figure 36).

<u>Male</u>
As stated earlier, there was no clear relationship between size of male American Eel and probability of being mature. This relationship held during our multivariate investigations as well, with the only significant covariate related to probability of maturity being capture season, with males exhibiting the highest probability of being mature when captured during the October through December period (Figure 37).

Age-at-maturity
<u>Female</u>
The best fit multiple regression model for female age-at-maturity dropped the covariates season and location from the final model. All other covariates (total length and presence of adult *A. crassus* in swim bladder) were retained. Like size-at-maturity, the effect of the presence of adult *A. crassus* in the swim bladder was clear, leading to earlier maturity of female American Eels when infected by the parasite (Figure 38).

<u>Male</u>
While the underlying curves were noisy, the multiple regression logistic models suggested all considered covariates significantly affected the probability of a male American Eel being mature, as all covariates were retained in the final model. With regards to location, the clearest effect was that male American Eels collected in the Winyah Bay system tended to mature at older ages, particularly when comparing to the maturity ogives of eels collected from the Ashley River and Edisto River (Figure 39). There was also a clear effect of season on probability of a male American Eel being mature with a given annulus count, with those captured from October-December maturing at an earlier age than those captured in other months (Figure 40). Finally, as with all other cases where the presence of *A. crassus* adults in the swim bladder was significant, the effect of presence was to induce earlier maturity of American Eel males.

<u>Significant Deviations</u>
None

Budget Report
A separate financial report will be submitted by the grant's administrator.

Recommendations
Close the grant

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Tables

Table 1: Precision metrics as calculated for three different comparisons: 1) between readers when annulus counts were determined using OTS method prepared ageing structures, 2) between readers when annulus counts were determined using WO method prepared ageing structures, & 3) comparison of consensus ages determined using the two, independent methods. Provided are the sample sizes (n), absolute percent agreement (% agreement), average coefficient of variation (ACV), average percent error (APE), and results of three symmetry tests (McNemar, Evans & Hoenig, & Bowker). For the symmetry test we provide the Chi-squared test statistic (χ^2), test degrees of freedom (DF), and calculated p-value of the symmetry test.

					Symmetry Tests								
					McNemar			Evans & Hoenig			Bowker		
Comparison	n	% Agreement	ACV	APE	χ^2	DF	P-value	χ^2	DF	P-value	χ^2	DF	P-value
OTS Method between Reader	1074	69.27%	7.99%	5.65%	1.212	1	0.2709	6.414	5	0.2680	46.57	34	0.0739
WO Method between Reader	1089	42.61%	25.34%	17.92%	105.7	1	<0.0001	114.6	6	<0.0001	138.9	37	<0.0001
Between Method Consensus	946	44.29%	20.08%	14.20%	225.9	1	<0.0001	241.0	9	<0.0001	254.9	51	<0.0001

Table 2: Whole otolith processing & ageing annulus count to otolith thin section processing & ageing annulus count conversion table. Annuli (1st column) represent annulus count based on whole otolith technique. Shown is the percentage of fish aged via the whole otolith technique assigned a given annulus count when aged via the otolith thin-section technique (row). Rows sum to 100%. The diagonal (outlined boxes) represents the 1 to 1 conversion line.

		Otolith Thin-Section Processing & Ageing Annulus Count													
Annuli	**American Eels**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
0	51	**35.3%**	19.6%	27.5%	5.9%	2.0%	2.0%	3.9%			2.0%	2.0%			
1	69		**43.5%**	26.1%	17.4%	4.3%	4.3%	1.4%	2.9%						
2	143		4.2%	**42.0%**	28.7%	14.7%	6.3%	3.5%			0.7%				
3	217		0.5%	5.5%	**51.6%**	24.9%	12.0%	2.3%	1.4%	0.5%	0.5%	0.9%			
4	201		0.5%		10.9%	**41.8%**	31.8%	11.4%	2.5%	0.0%	1.0%				
5	148				2.0%	11.5%	**51.4%**	20.9%	6.8%	6.1%	0.7%			0.7%	
6	69				1.4%	1.4%	14.5%	**42.0%**	21.7%	11.6%	4.3%	2.9%		0.0%	
7	29					6.9%	6.9%	24.1%	**31.0%**	10.3%	10.3%	6.9%		3.4%	
8	13							23.1%	7.7%	**7.7%**	30.8%	15.4%	7.7%	7.7%	
9	5					20.0%						20.0%	40.0%		20.0%
10	-														
11	-														
12	1												100.0%		

Table 3: Number of American Eels with different types of associated biological and environmental data recorded since 2010. Not every American Eel had all parameters recorded.

Parameter	n
Biological Parameter	
Total Length (mm)	1,604
Weight (g)	1,604
Life Stage (Yellow Eel or Silver Eel)	1,601
Swim bladder Impairment	1,541
Presence of Adult *A. crassus*	1,594
# of Adult *A. crassus*	1,594
Sex (Macroscopic)	1,612
Sex (Histologically)	1,141
Maturity	1,125
Female Maturity	385
Max Oocyte Diameter	794
Annuli (OTS Method)	1,374
Annuli (WO Method)	1,081
Age (OTS Method)	1,374
Age (WO Method)	1,081
Environmental Parameter	
Salinity (PSU)	1,524
Water Temperature (^{o}C)	1,525
Dissolved Oxygen (mg/L)	1,444

Table 4: Raw age-length key developed using OTS method derived annulus counts. This age-length key pools information across all years (2010-2019).

		Annulus Count													
Length (mm)	**Fish**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
80-99	**2**	1.000													
100-119	**2**	0.500	0.500												
120-139	**14**	0.643	0.214	0.071				0.071							
140-159	**27**	0.333	0.444	0.185	0.037										
160-179	**29**	0.138	0.379	0.276	0.138	0.034	0.034								
180-199	**41**	0.146	0.244	0.488	0.098	0.024									
200-219	**45**	0.089	0.289	0.311	0.222	0.022	0.022	0.022	0.022						
220-239	**33**	0.030	0.152	0.424	0.182	0.152	0.030	0.030							
240-259	**45**		0.089	0.378	0.356	0.067	0.067	0.022				0.022			
260-279	**66**		0.045	0.258	0.424	0.182	0.061	0.015	0.015						
280-299	**96**		0.042	0.208	0.292	0.281	0.125	0.042				0.010			
300-319	**94**		0.021	0.106	0.287	0.266	0.191	0.053	0.021	0.011	0.011	0.021	0.011		
320-339	**102**	0.010	0.029	0.098	0.294	0.255	0.127	0.108	0.049	0.020	0.010				
340-359	**62**		0.016	0.081	0.290	0.242	0.145	0.113	0.048	0.016	0.016	0.016			0.016
360-379	**48**		0.021	0.063	0.292	0.250	0.292	0.042	0.021		0.021				
380-399	**36**			0.139	0.222	0.333	0.194	0.083	0.028						
400-419	**38**			0.053	0.211	0.500	0.132	0.053	0.053						
420-439	**61**		0.016	0.016	0.230	0.262	0.328	0.131	0.016						
440-459	**61**		0.016	0.016	0.164	0.262	0.328	0.131	0.016	0.016			0.033		0.016
460-479	**89**		0.011	0.022	0.135	0.225	0.382	0.157	0.067						
480-499	**67**			0.030	0.119	0.284	0.299	0.164	0.060	0.045					
500-519	**81**			0.012	0.074	0.210	0.321	0.148	0.160	0.049	0.012		0.012		
520-539	**58**			0.000	0.138	0.224	0.224	0.276	0.034	0.086	0.017				
540-559	**38**			0.026	0.026	0.289	0.158	0.316	0.105	0.026	0.053				
560-579	**39**	0.026		0.026	0.077	0.103	0.256	0.333	0.051	0.077	0.026	0.026			
580-599	**32**			0.063	0.031	0.156	0.125	0.250	0.125	0.125	0.063	0.063			
600-619	**25**					0.120	0.240	0.240	0.160	0.040	0.080	0.040	0.040	0.040	
620-639	**15**			0.067	0.067		0.200	0.333	0.200	0.067		0.067			
640-659	**10**					0.200	0.300		0.200		0.200	0.100			
660-679	**4**				0.250	0.250			0.250	0.250					
680-699	**3**						0.333		0.667						
700-719	**5**							0.200	0.400	0.200	0.200				
720-739	**2**					0.500				0.500					
740-759	**1**						1.000								
760-779	**1**									1.000					
780-799	**1**									1.000					
800-819	**0**														
820-839	**0**														
840-859	**0**														
860-879	**0**														
880-899	**0**														
900-919	**1**											1.000			

Table 5: Smoothed age-length key developed using OTS method derived annulus counts. Smoothing accomplished using multinomial logistic regression. This age-length key pools information across all years (2010-2019).

	Annulus Count													
Length (mm)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
80-99	0.634	0.253	0.086	0.021	0.005	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100-119	0.534	0.291	0.125	0.036	0.009	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120-139	0.426	0.317	0.173	0.059	0.017	0.006	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
140-159	0.318	0.323	0.223	0.091	0.029	0.011	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
160-179	0.222	0.307	0.269	0.130	0.047	0.019	0.005	0.001	0.000	0.000	0.000	0.000	0.000	0.000
180-199	0.143	0.271	0.301	0.174	0.070	0.030	0.009	0.002	0.000	0.000	0.000	0.000	0.000	0.001
200-219	0.087	0.223	0.314	0.216	0.097	0.044	0.013	0.003	0.000	0.000	0.000	0.001	0.000	0.001
220-239	0.049	0.173	0.309	0.254	0.126	0.061	0.020	0.005	0.001	0.001	0.001	0.001	0.000	0.001
240-259	0.027	0.127	0.288	0.282	0.156	0.080	0.027	0.007	0.001	0.001	0.001	0.001	0.000	0.001
260-279	0.014	0.090	0.258	0.300	0.185	0.101	0.036	0.009	0.002	0.001	0.002	0.001	0.000	0.001
280-299	0.007	0.061	0.222	0.308	0.211	0.123	0.047	0.013	0.002	0.002	0.002	0.002	0.000	0.002
300-319	0.003	0.040	0.185	0.306	0.233	0.145	0.058	0.017	0.003	0.002	0.003	0.002	0.000	0.002
320-339	0.002	0.026	0.150	0.297	0.252	0.167	0.071	0.021	0.005	0.003	0.004	0.003	0.000	0.002
340-359	0.001	0.016	0.120	0.282	0.265	0.187	0.084	0.026	0.006	0.004	0.004	0.003	0.000	0.002
360-379	0.000	0.010	0.093	0.262	0.275	0.206	0.097	0.032	0.008	0.005	0.005	0.003	0.000	0.002
380-399	0.000	0.006	0.072	0.240	0.279	0.224	0.111	0.039	0.011	0.007	0.007	0.004	0.000	0.002
400-419	0.000	0.004	0.054	0.216	0.280	0.239	0.126	0.046	0.014	0.008	0.008	0.004	0.000	0.002
420-439	0.000	0.002	0.040	0.192	0.277	0.251	0.140	0.053	0.017	0.010	0.009	0.005	0.000	0.002
440-459	0.000	0.001	0.030	0.169	0.271	0.262	0.154	0.062	0.022	0.012	0.011	0.005	0.000	0.002
460-479	0.000	0.001	0.022	0.147	0.262	0.270	0.167	0.070	0.027	0.015	0.012	0.005	0.000	0.002
480-499	0.000	0.000	0.016	0.126	0.251	0.275	0.180	0.079	0.033	0.018	0.014	0.006	0.001	0.002
500-519	0.000	0.000	0.011	0.107	0.238	0.277	0.192	0.089	0.040	0.021	0.016	0.006	0.001	0.002
520-539	0.000	0.000	0.008	0.091	0.223	0.277	0.202	0.098	0.048	0.024	0.018	0.006	0.001	0.001
540-559	0.000	0.000	0.006	0.076	0.208	0.275	0.212	0.108	0.058	0.028	0.020	0.007	0.002	0.001
560-579	0.000	0.000	0.004	0.063	0.192	0.270	0.220	0.118	0.068	0.033	0.022	0.007	0.002	0.001
580-599	0.000	0.000	0.003	0.052	0.176	0.264	0.227	0.127	0.080	0.037	0.024	0.007	0.003	0.001
600-619	0.000	0.000	0.002	0.042	0.160	0.255	0.232	0.136	0.094	0.042	0.026	0.007	0.003	0.001
620-639	0.000	0.000	0.001	0.034	0.144	0.245	0.235	0.145	0.108	0.047	0.027	0.007	0.004	0.001
640-659	0.000	0.000	0.001	0.028	0.129	0.234	0.236	0.153	0.124	0.053	0.029	0.007	0.006	0.001
660-679	0.000	0.000	0.001	0.022	0.115	0.221	0.236	0.160	0.141	0.058	0.031	0.007	0.007	0.001
680-699	0.000	0.000	0.000	0.017	0.101	0.208	0.234	0.167	0.160	0.064	0.032	0.007	0.009	0.001
700-719	0.000	0.000	0.000	0.014	0.089	0.193	0.230	0.172	0.179	0.070	0.034	0.006	0.011	0.001
720-739	0.000	0.000	0.000	0.011	0.077	0.179	0.225	0.177	0.200	0.076	0.035	0.006	0.014	0.000
740-759	0.000	0.000	0.000	0.008	0.067	0.165	0.218	0.180	0.221	0.082	0.036	0.006	0.017	0.000
760-779	0.000	0.000	0.000	0.006	0.057	0.150	0.211	0.182	0.243	0.087	0.036	0.006	0.020	0.000
780-799	0.000	0.000	0.000	0.005	0.049	0.136	0.202	0.183	0.265	0.093	0.037	0.005	0.025	0.000
800-819	0.000	0.000	0.000	0.004	0.041	0.123	0.192	0.183	0.288	0.098	0.037	0.005	0.030	0.000
820-839	0.000	0.000	0.000	0.003	0.035	0.110	0.181	0.181	0.310	0.102	0.037	0.005	0.035	0.000
840-859	0.000	0.000	0.000	0.002	0.029	0.098	0.170	0.179	0.332	0.107	0.037	0.004	0.042	0.000
860-879	0.000	0.000	0.000	0.002	0.024	0.086	0.159	0.175	0.354	0.110	0.037	0.004	0.049	0.000
880-899	0.000	0.000	0.000	0.001	0.020	0.076	0.147	0.170	0.374	0.113	0.036	0.004	0.058	0.000
900-919	0.000	0.000	0.000	0.001	0.016	0.066	0.136	0.165	0.394	0.116	0.035	0.003	0.067	0.000

Table 6: von Bertalanffy growth curve parameter estimates. Provided are parameter estimates and 95% CI (based on 10,000 bootstraps) for growth models developed using sexes pooled, male only, and female only data using either the OTS method or WO method ageing protocols.

			L_∞			k			t_0		
Model	**Ageing Method**	**n**	**Estimate**	**LCI**	**UCI**	**Estimate**	**LCI**	**UCI**	**Estimate**	**LCI**	**UCI**
Sexes Pooled	OTS	1374	619.3	567.1	700.2	0.1943	0.1445	0.2470	-1.298	-1.824	-0.913
Male	OTS	343	358.9	332.1	406.7	0.2985	0.1905	0.4212	-1.907	-2.923	-1.289
Female	OTS	865	654.3	594.6	754.7	0.1940	0.1384	0.2531	-1.187	-1.777	-0.755
Sexes Pooled	WO	1074	830.9	694.3	1147.2	0.1297	0.0747	0.1872	-1.704	-2.337	-1.246
Male	WO	232	441.2	333.4	1057.4	0.1863	0.0393	0.4816	-2.962	-5.451	-1.453
Female	WO	720	715.6	631.3	880.5	0.1856	0.1200	0.2531	-1.368	-1.986	-0.931

Table 7: Logistic regression model parameter estimates. Provided are parameter estimates and 95% CI (based on 10,000 bootstraps) for maturity ogives developed using sex specific data estimating probability of maturity at a given size (total length, in mm) and age (annulus count).

Variable			**B0**			**B1**		
Dependent	**Independent**	**Sex**	**Estimate**	**Lower CI**	**Upper CI**	**Estimate**	**Lower CI**	**Upper CI**
Maturity	Total Length (mm)	Female	-13.96	-16.71	-11.92	0.0310	0.0267	0.0369
Maturity*	Total Length (mm)	Male	-2.68	-6.68	0.93	0.0108	-0.0007	0.0238
Maturity	Annulus Count	Female	-2.60	-3.27	-2.01	0.6123	0.4861	0.7564
Maturity	Annulus Count	Male	-0.76	-1.82	0.12	0.3350	0.1472	0.5926

* – Note, this model suggested there was not a relationship between size and probability of maturity.

Table 8: Estimated length- and age-at-50% maturity and 90% maturity based on best fit logistic regressions. Provided are best estimate along with 95% CI of parameter estimates based on 10,000 bootstraps.

Parameter	Sex	Estimate	Lower CI	Upper CI
Length-@-50% Maturity	Female	450.3	442.5	457.7
Length-@-90% Maturity	Female	521.1	509.4	533.1
Length-@-50% Maturity*	Male	248.5	-144.6	497.3
Length-@-90% Maturity*	Male	452.0	-123.5	1301.5
Annulus Count-@-50% Maturity	Female	4.2	4.0	4.5
Annulus Count-@-90% Maturity	Female	7.8	7.1	8.7
Annulus Count-@-50% Maturity	Male	2.3	-0.7	3.4
Annulus Count-@-90% Maturity	Male	8.8	6.5	14.6

* – Note, this model suggested there was not a relationship between size and probability of maturity.

Figures

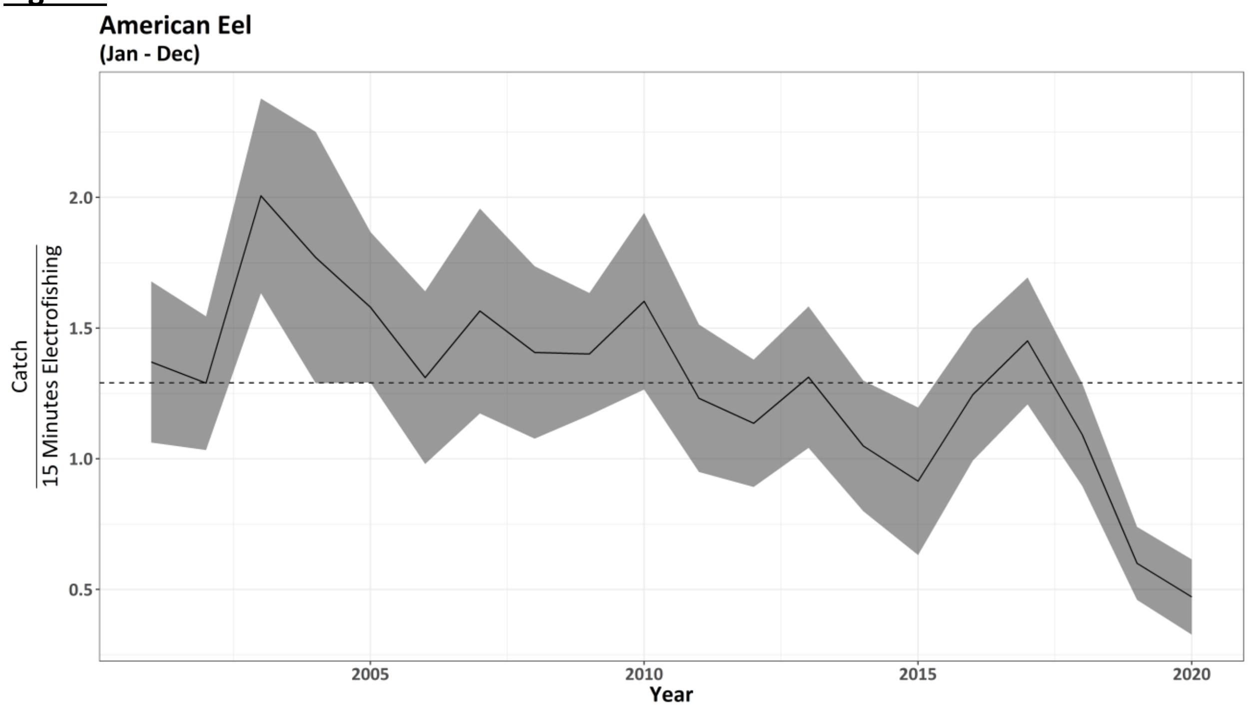

Figure 1: American eel abundance as observed in the South Carolina Department of Natural Resources statewide electrofishing survey of upper estuary habitats. Note 2020 catches were impacted by sampling interruptions due to the COVID-19 pandemic, though magnitude of impact on American Eel catches appears minor based on comparisons with historic data (J. Ballenger, pers. comm.).

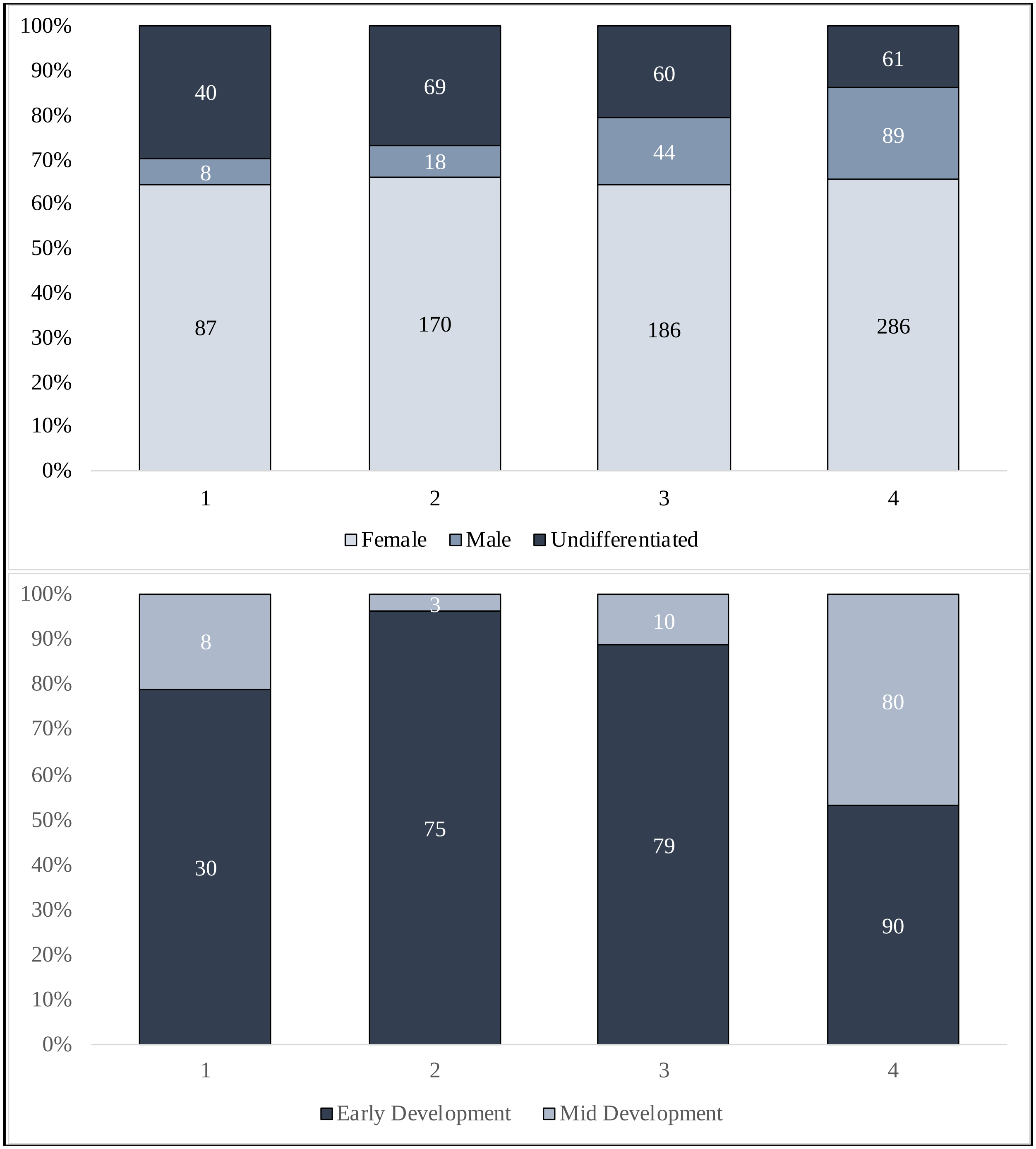

Figure 2: By quarter (January-March, April-June, July-September, & October-December), (top) proportion of undifferentiated, female, and male American Eels and (bottom) female American eels classified as developing showing characteristics of early- and mid-development stage.

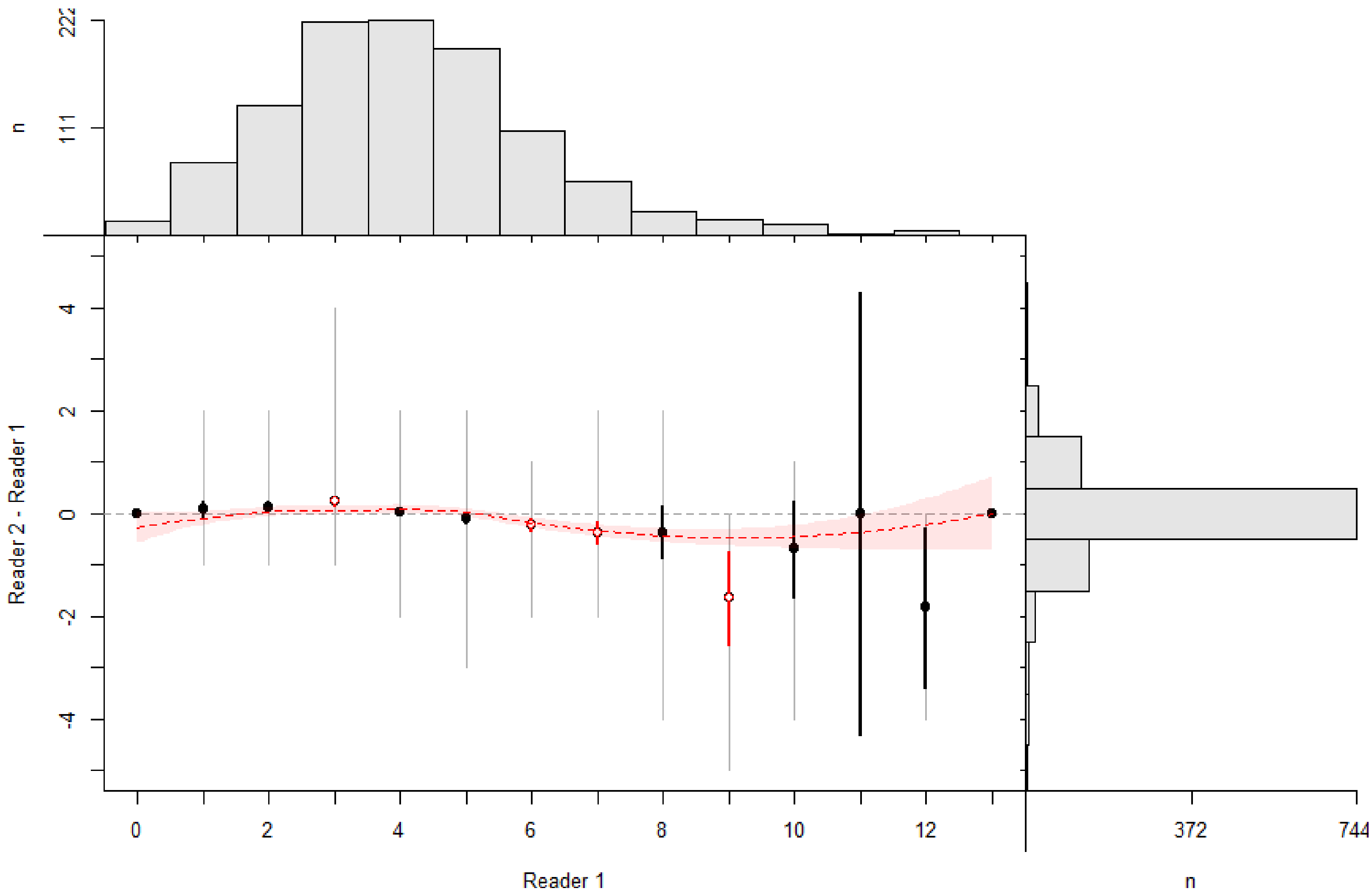

Figure 3: Age bias estimates between readers using the OTS ageing method. Shown is the annulus distribution of age determinations by Reader 1 (top histogram), histogram of differences in annulus counts between readers (right histogram), and the mean age difference (circles), 95% confidence interval of age difference (heavy black/red lines) and range of age differences (gray vertical lines) for each Reader 1 reference annulus count. Open, red circles with red confidence interval lines indicate significant bias in annulus counts between readers based on 95% confidence interval of mean difference not containing zero. Also shown is a LOESS smoother (dashed red line) with 95% CI (shaded red area) of mean difference as a function of reference annulus count.

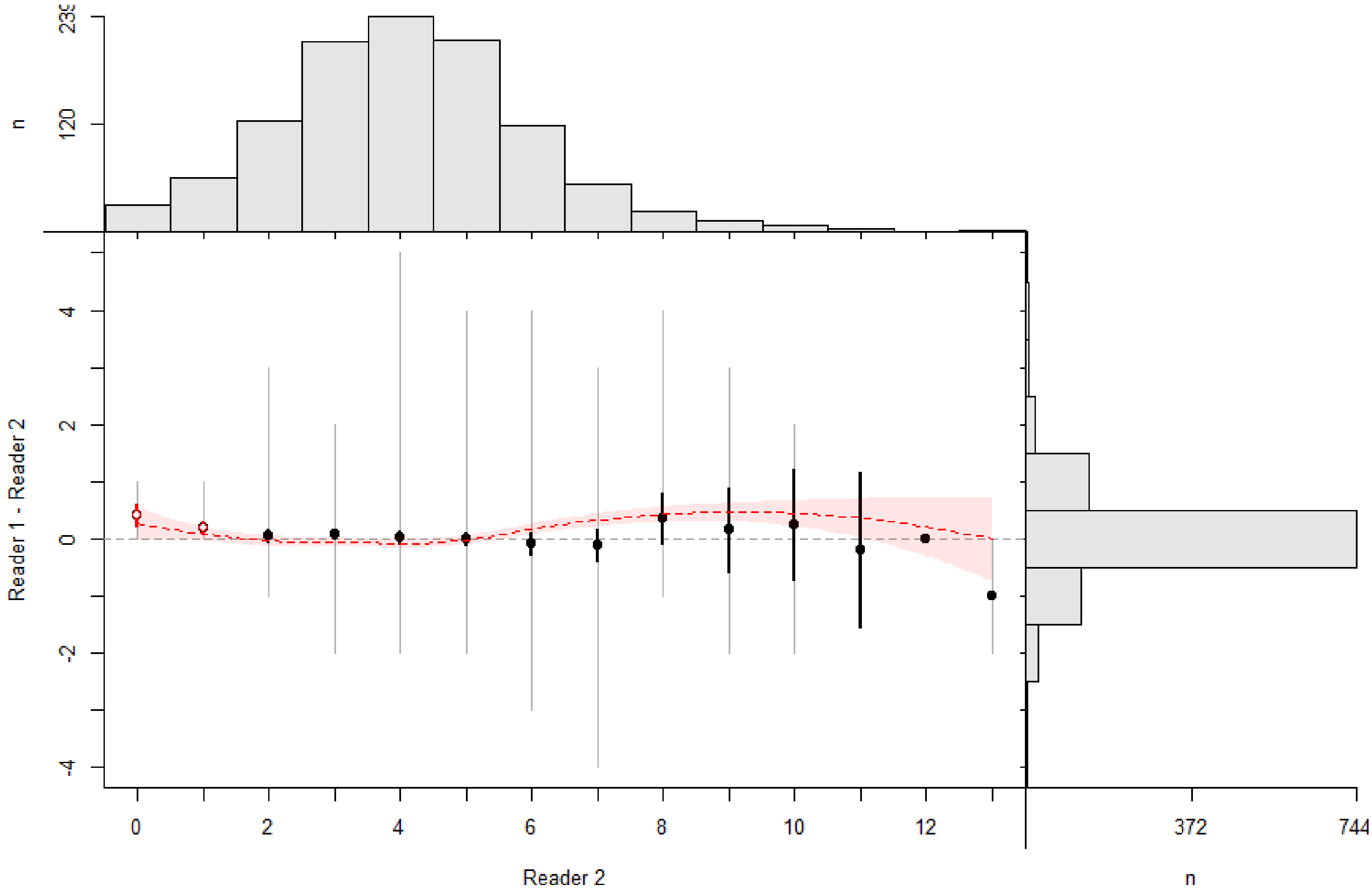

Figure 4: Age bias estimates between readers using the OTS ageing method. Shown is the annulus distribution of age determinations by Reader 2 (top histogram), histogram of differences in annulus counts between readers (right histogram), and the mean age difference (circles), 95% confidence interval of age difference (heavy black/red lines) and range of age differences (gray vertical lines) for each Reader 2 reference annulus count. Open, red circles with red confidence interval lines indicate significant bias in annulus counts between readers based on 95% confidence interval of mean difference not containing zero. Also shown is a LOESS smoother (dashed red line) with 95% CI (shaded red area) of mean difference as a function of reference annulus count.

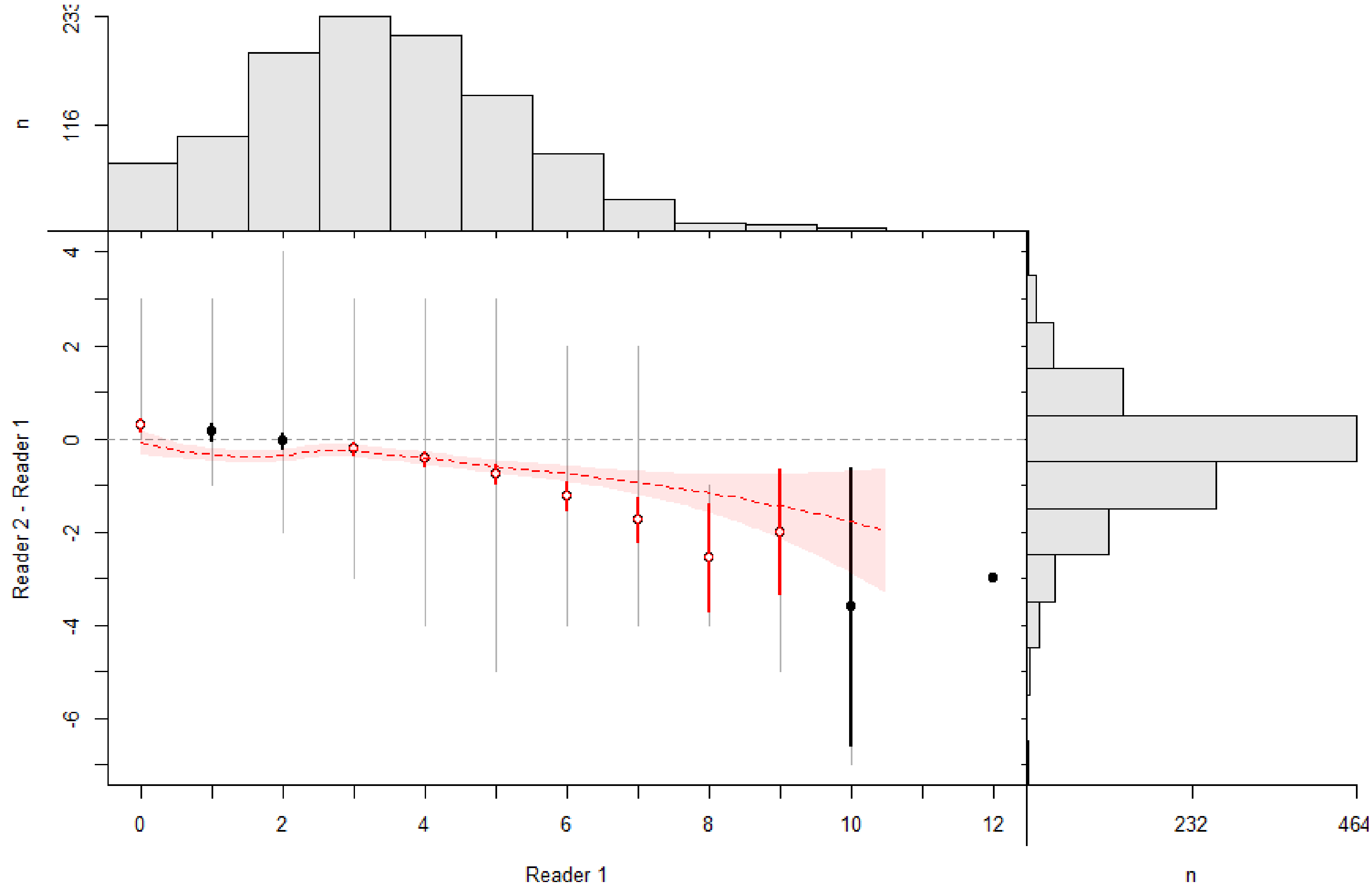

Figure 5: Age bias estimates between readers using the WO ageing method. Shown is the annulus distribution of age determinations by Reader 1 (top histogram), histogram of differences in annulus counts between readers (right histogram), and the mean age difference (circles), 95% confidence interval of age difference (heavy black/red lines) and range of age differences (gray vertical lines) for each Reader 1 reference annulus count. Open, red circles with red confidence interval lines indicate significant bias in annulus counts between readers based on 95% confidence interval of mean difference not containing zero. Also shown is a LOESS smoother (dashed red line) with 95% CI (shaded red area) of mean difference as a function of reference annulus count.

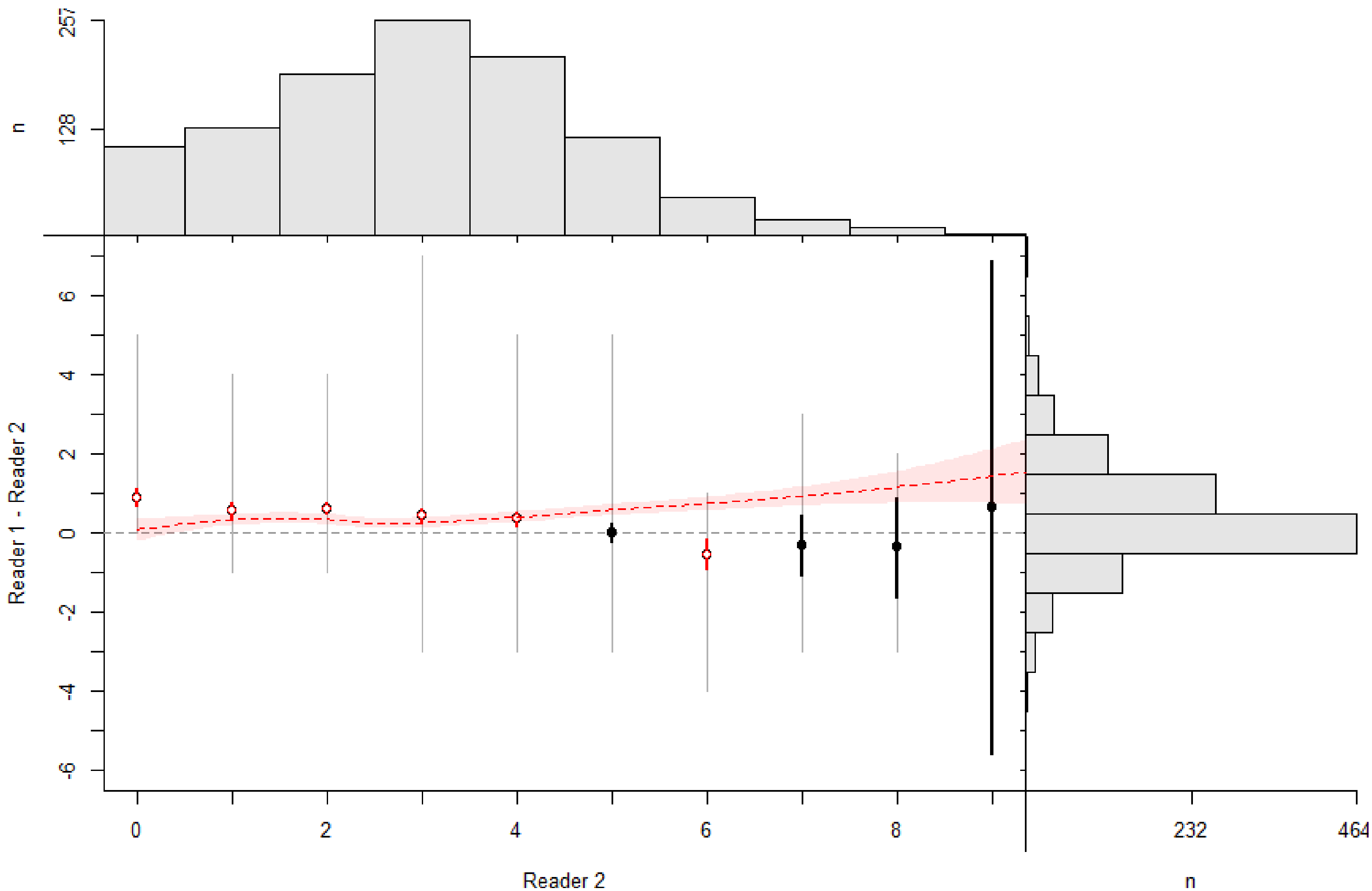

Figure 6: Age bias estimates between readers using the WO ageing method. Shown is the annulus distribution of age determinations by Reader 2 (top histogram), histogram of differences in annulus counts between readers (right histogram), and the mean age difference (circles), 95% confidence interval of age difference (heavy black/red lines) and range of age differences (gray vertical lines) for each Reader 2 reference annulus count. Open, red circles with red confidence interval lines indicate significant bias in annulus counts between readers based on 95% confidence interval of mean difference not containing zero. Also shown is a LOESS smoother (dashed red line) with 95% CI (shaded red area) of mean difference as a function of reference annulus count.

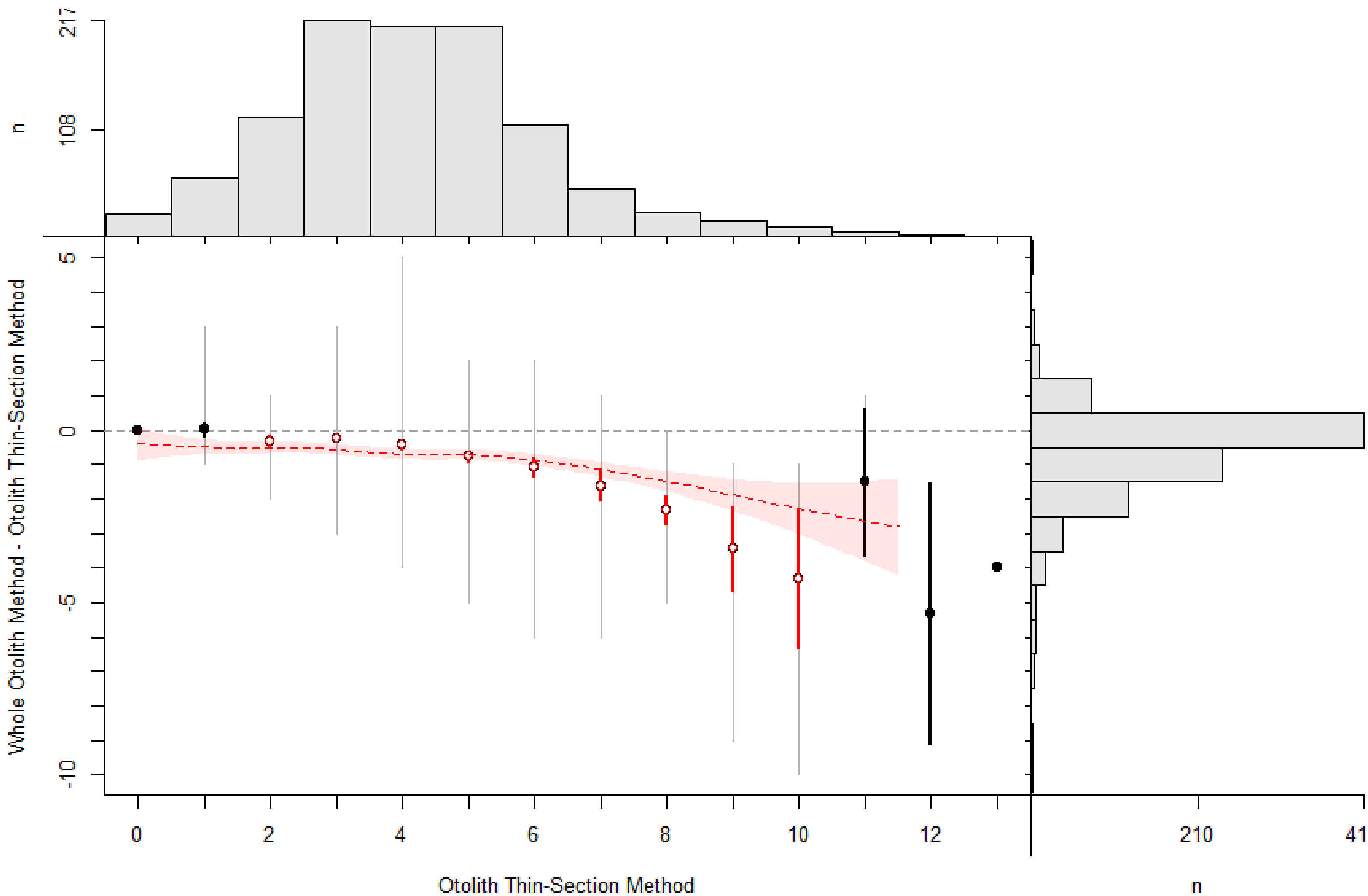

Figure 7: Age bias estimates between ageing methodologies based on consensus annulus counts. Shown is the annulus distribution of age determinations by the OTS method (top histogram), histogram of differences in annulus counts between methods (right histogram), and the mean age difference (circles), 95% confidence interval of age difference (heavy black/red lines) and range of age differences (gray vertical lines) for each OTS method reference annulus count. Open, red circles with red confidence interval lines indicate significant bias in annulus counts between ageing methods based on 95% confidence interval of mean difference not containing zero. Also shown is a LOESS smoother (dashed red line) with 95% CI (shaded red area) of mean difference as a function of reference annulus count.

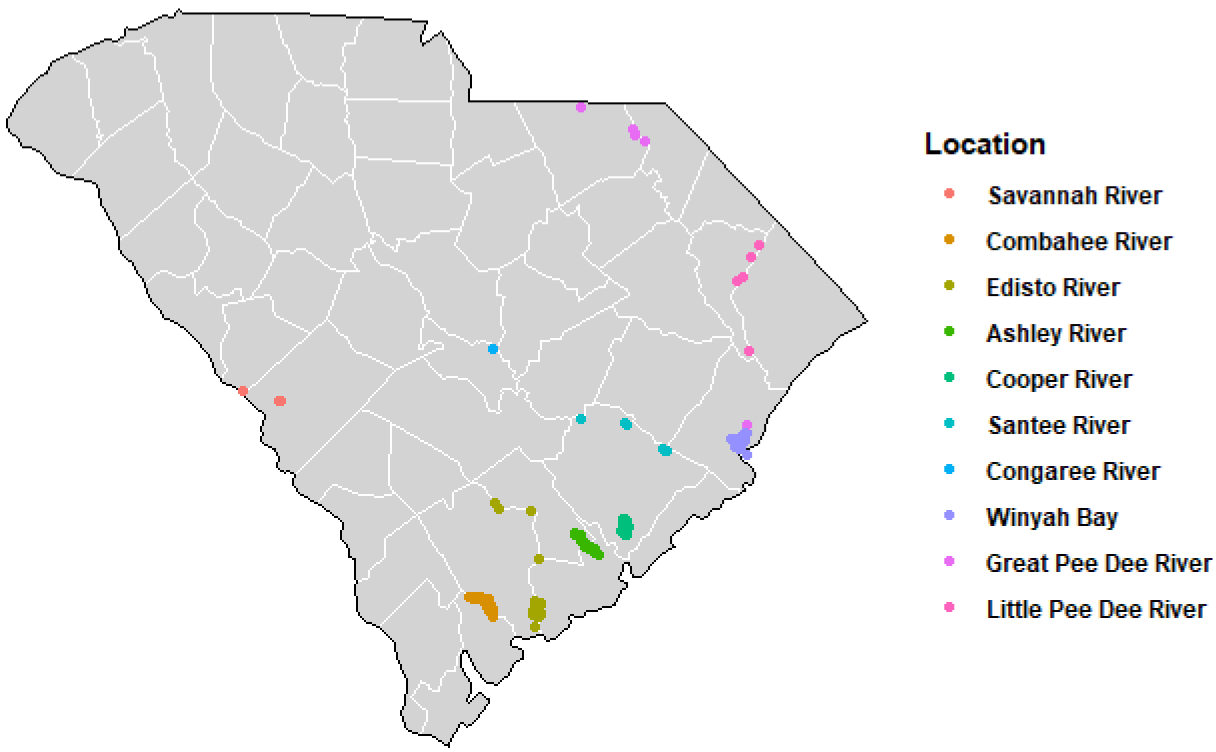

Figure 8: Locations where we have collected American Eels for life history analysis since 2010. Samples are color coded to their river/estuary of origin. Note, most samples derive from upper estuary, tidally influenced, low salinity areas sampled routinely by our fishery-independent survey in the Combahee River, Edisto River, Ashley River, Cooper River, and Winyah Bay.

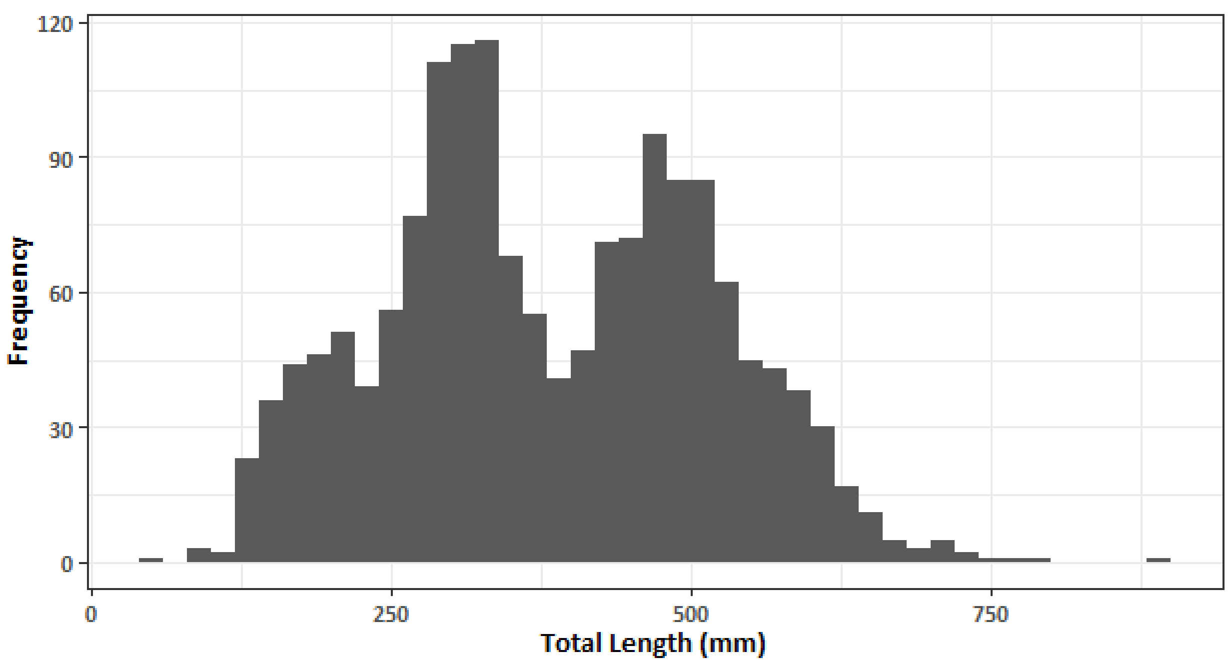

Figure 9: Length frequency of American Eels retained for detailed life history study by the SCDNR. Bins are 20 mm wide, ranging from 40 to 920 mm TL.

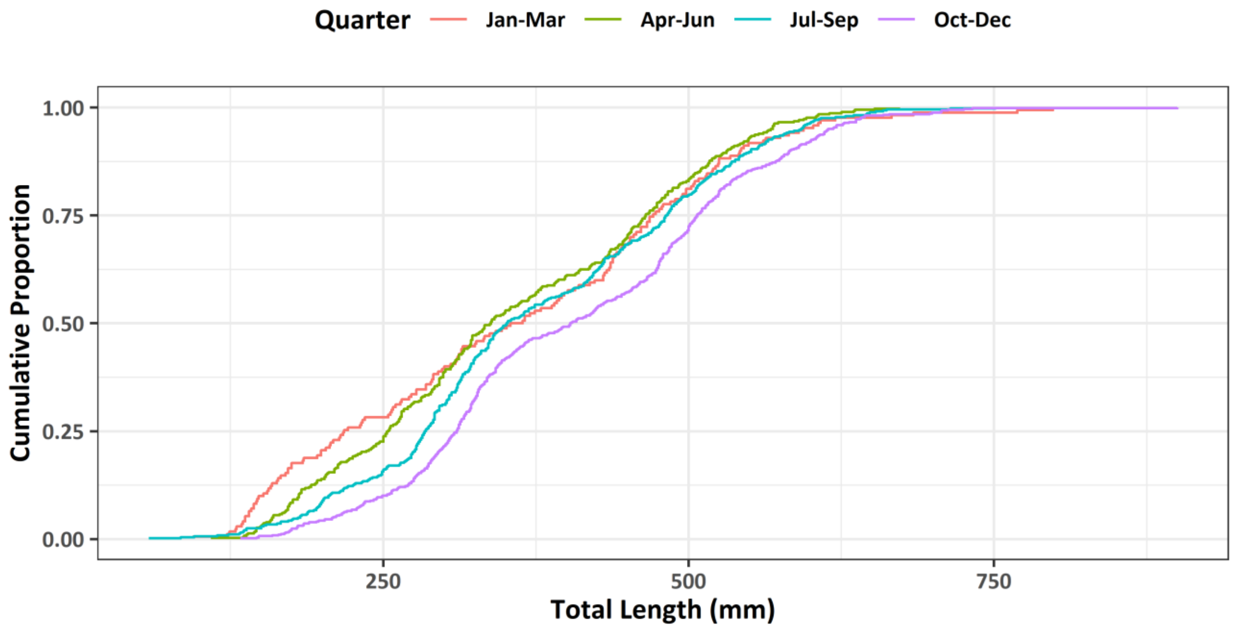

Figure 10: Empirical cumulative distribution function showing the sizes of American Eel retained for detailed life history information by quarter.

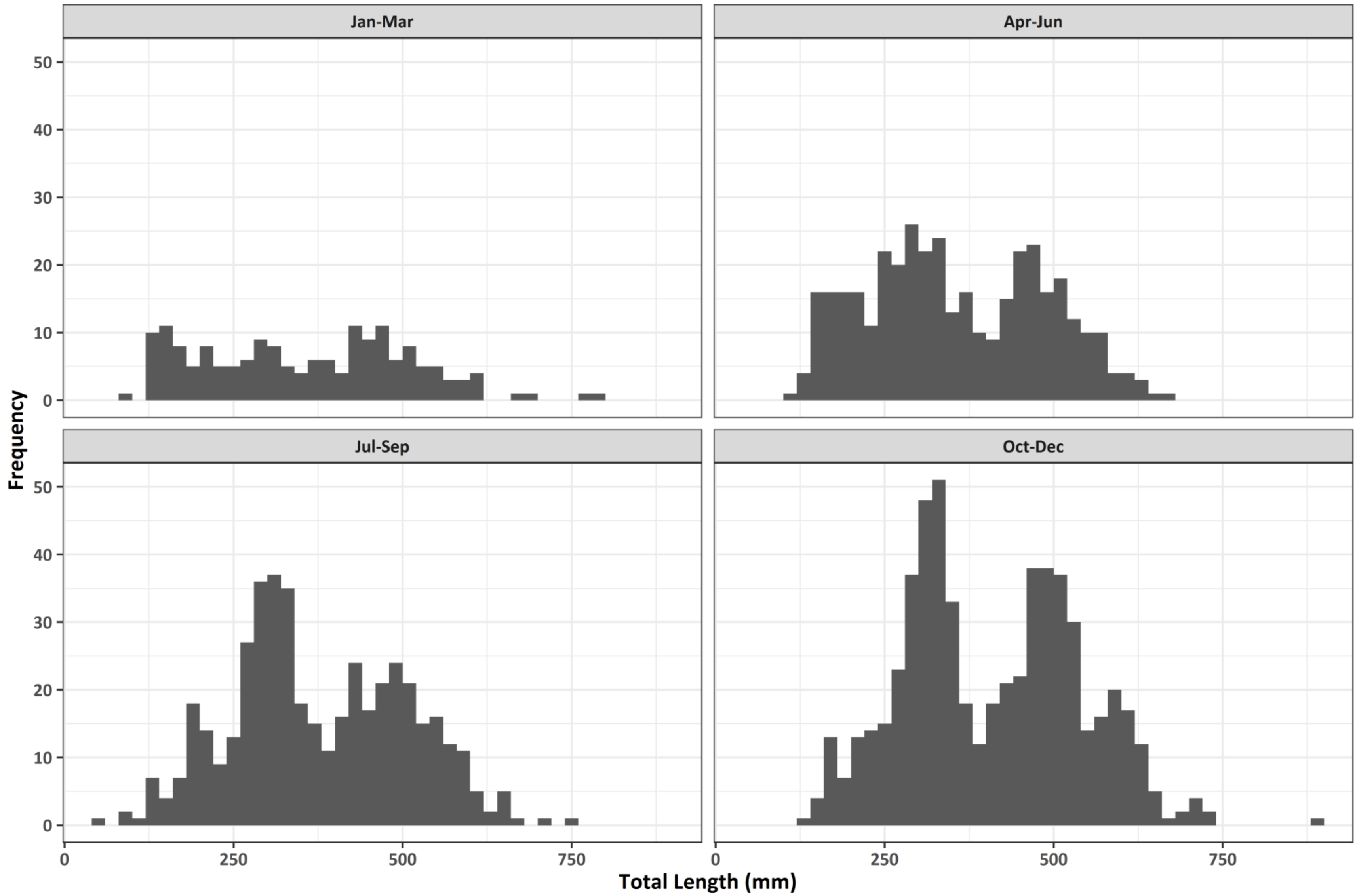

Figure 11: Length frequency of American Eels retained for detailed life history study by the SCDNR by quarter. Bins are 20 mm wide, ranging from 40 to 920 mm TL.

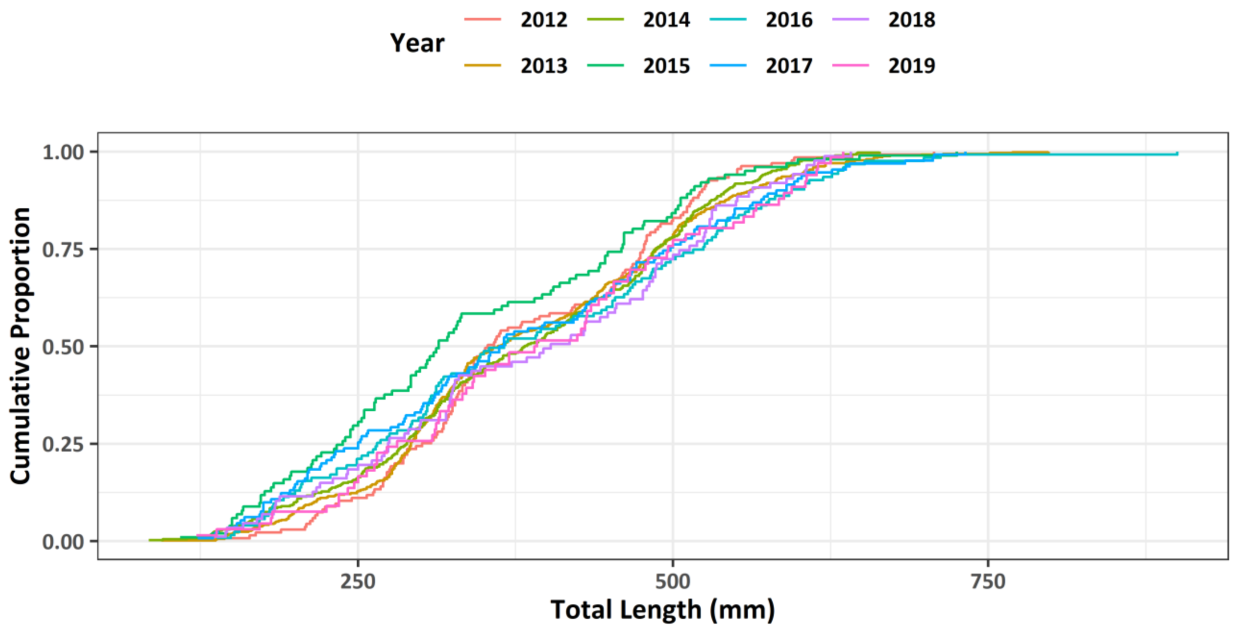

Figure 12: Empirical cumulative distribution function showing the sizes of American Eel retained for detailed life history information by year. Note, only years where a minimum of 30 individual American Eels were retained are shown.

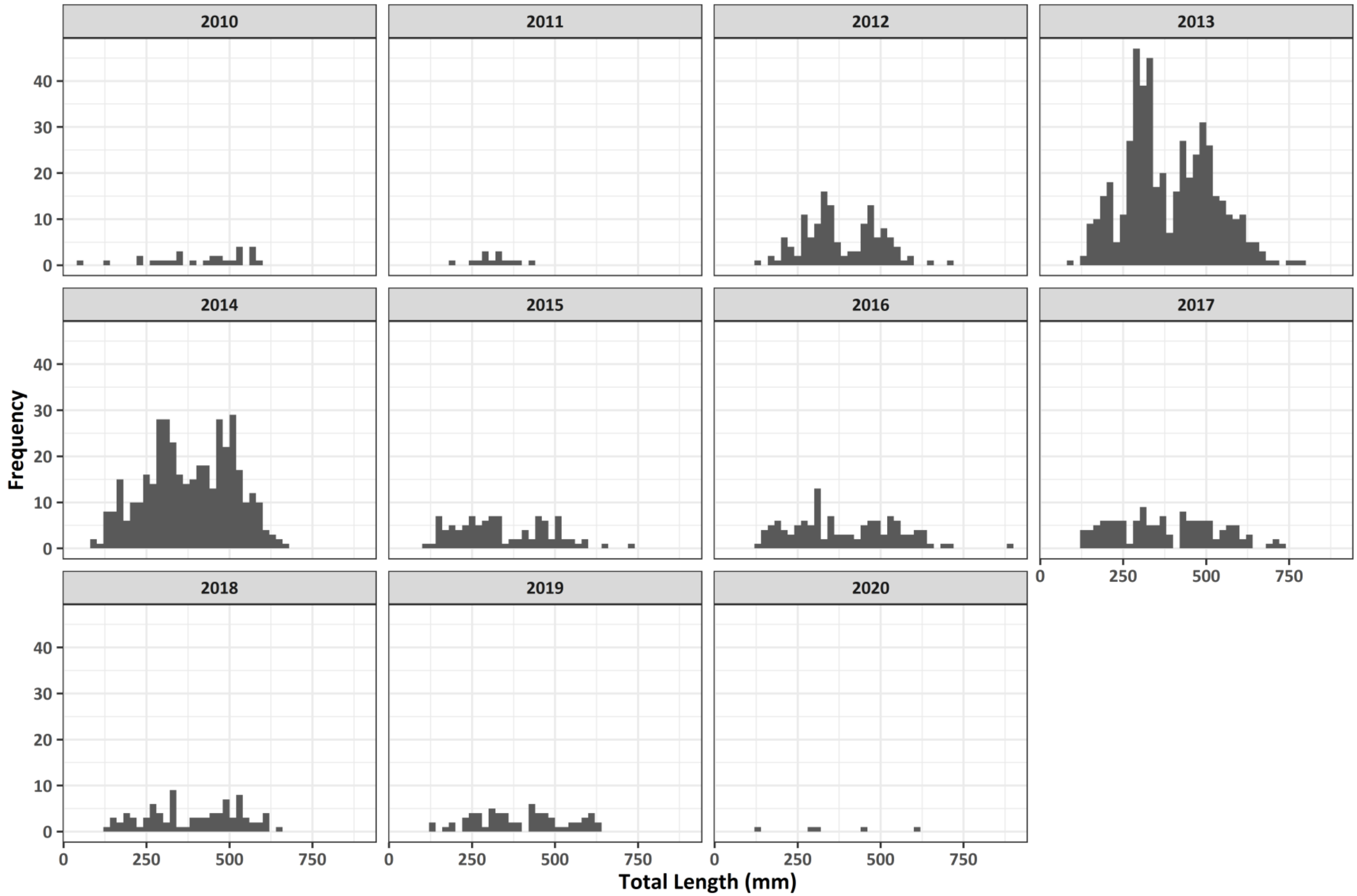

Figure 13: Length frequency of American Eels retained for detailed life history study by the SCDNR by year. Bins are 20 mm wide, ranging from 40 to 920 mm TL.

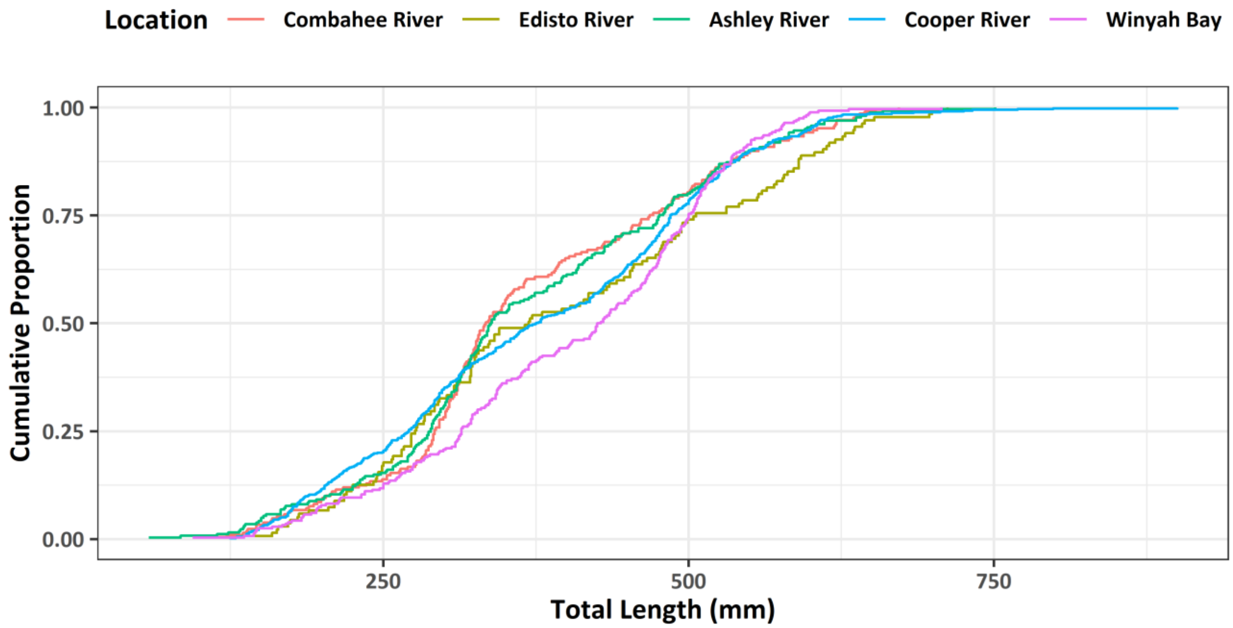

Figure 14: Empirical cumulative distribution function showing the sizes of American Eel retained for detailed life history information by electrofishing survey strata.

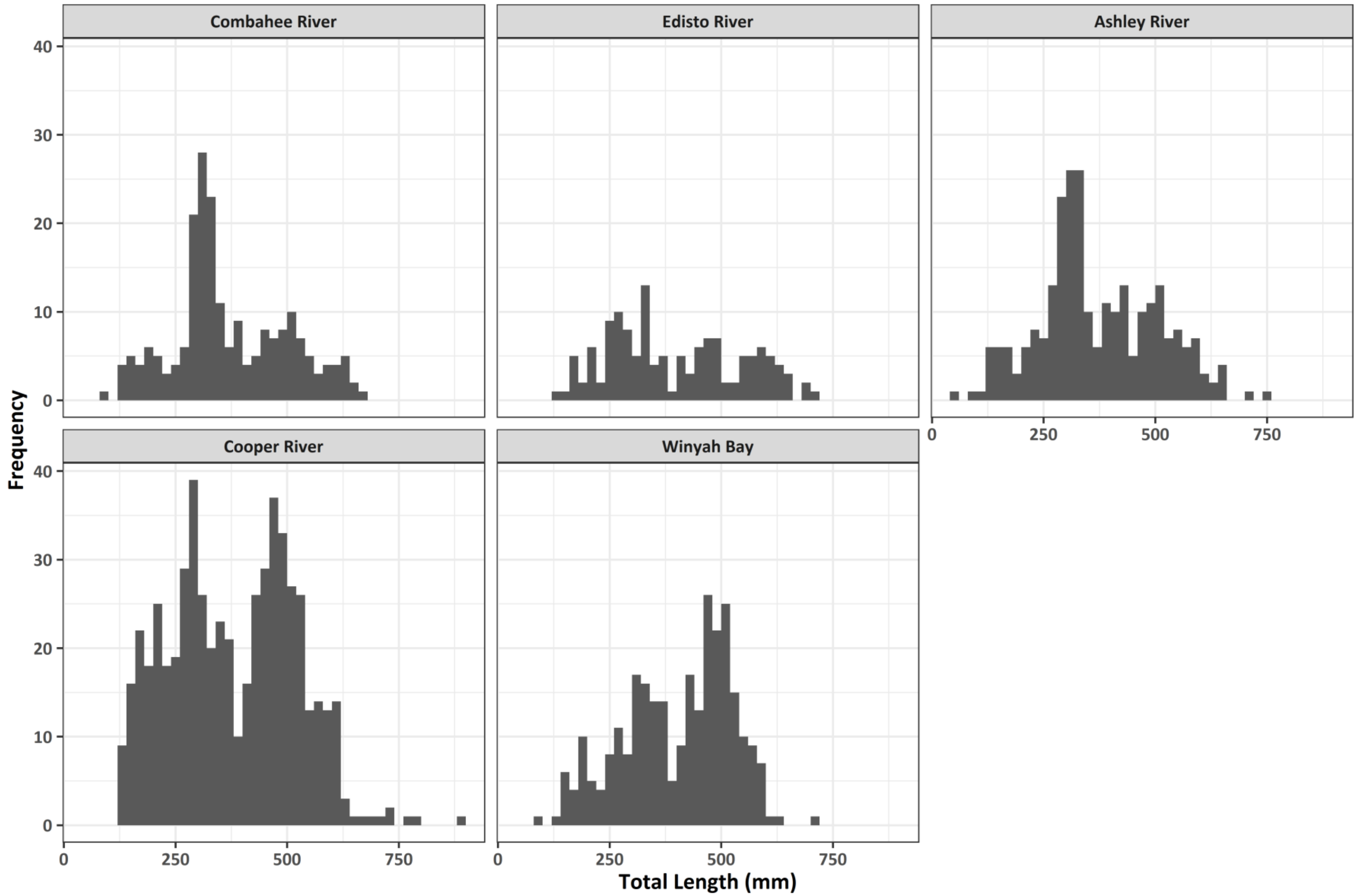

Figure 15: Length frequency of American Eels retained for detailed life history study by the SCDNR by electrofishing survey strata. Bins are 20 mm wide, ranging from 40 to 920 mm TL.

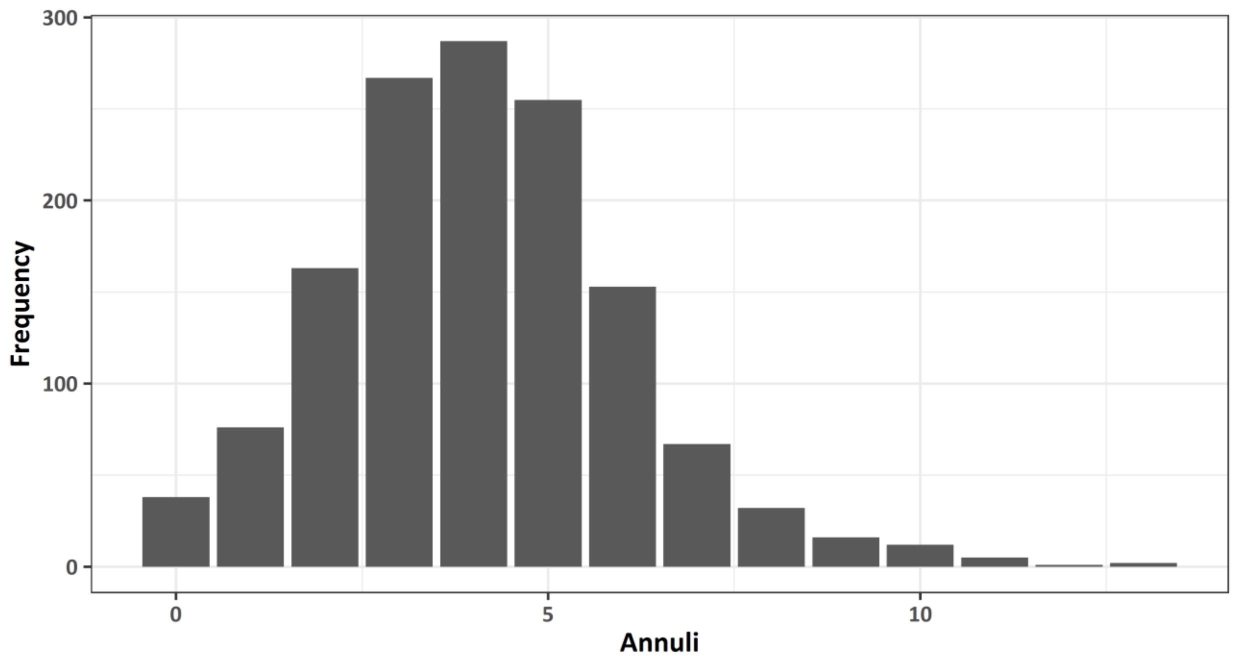

Figure 16: Annulus count composition of American Eels retained for detailed life history study by the SCDNR.

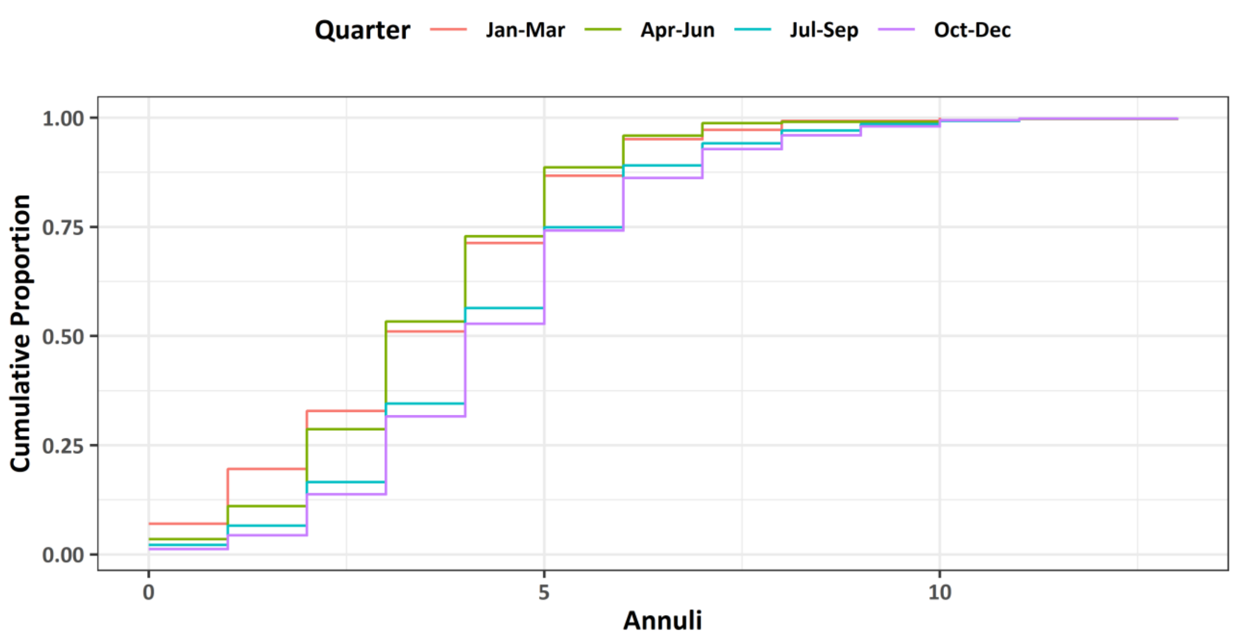

Figure 17: Empirical cumulative distribution function showing the annuli counts of American Eel retained for detailed life history information by quarter.

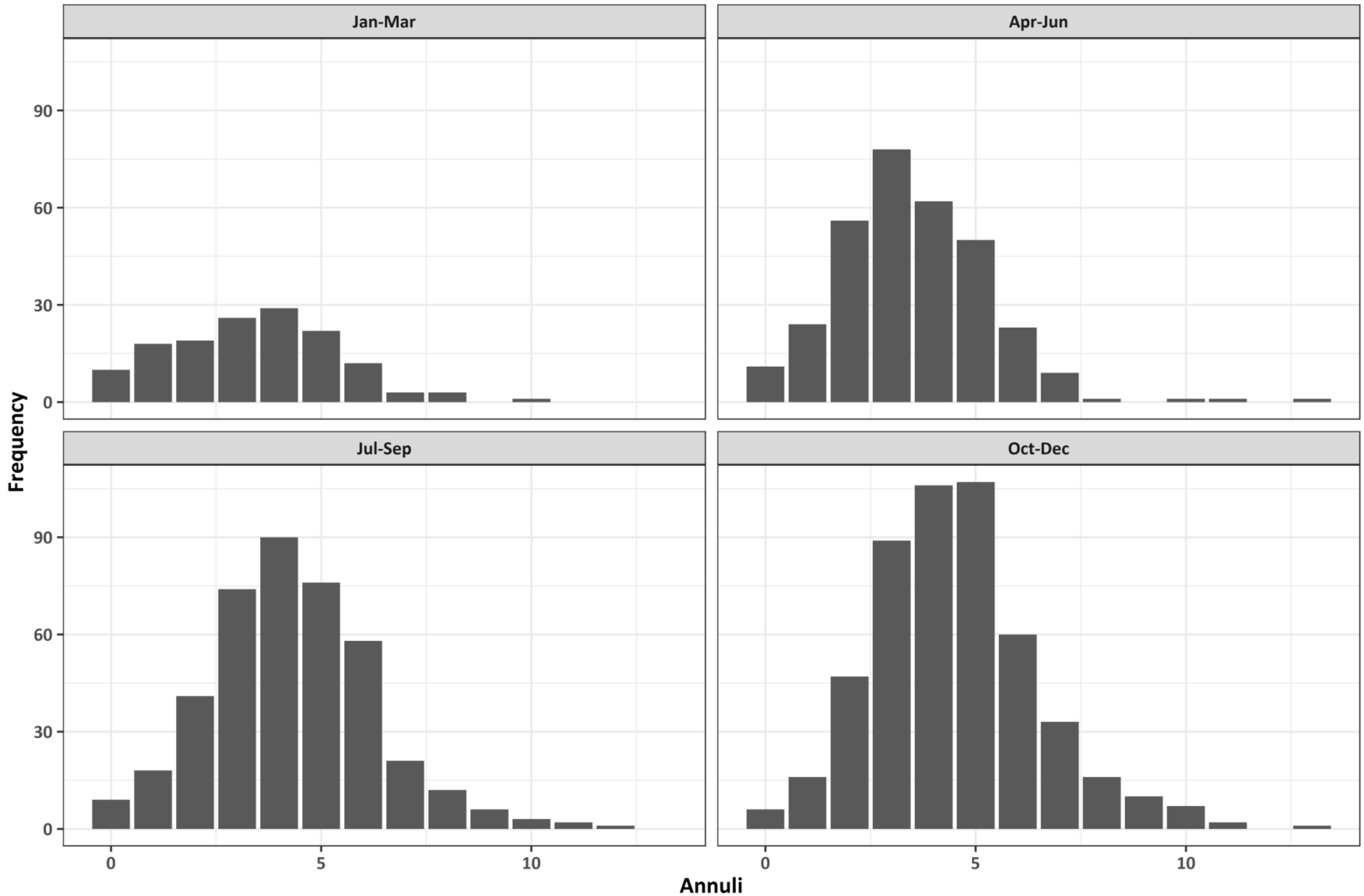

Figure 18: Annulus count composition of American Eels retained for detailed life history study by the SCDNR by quarter.

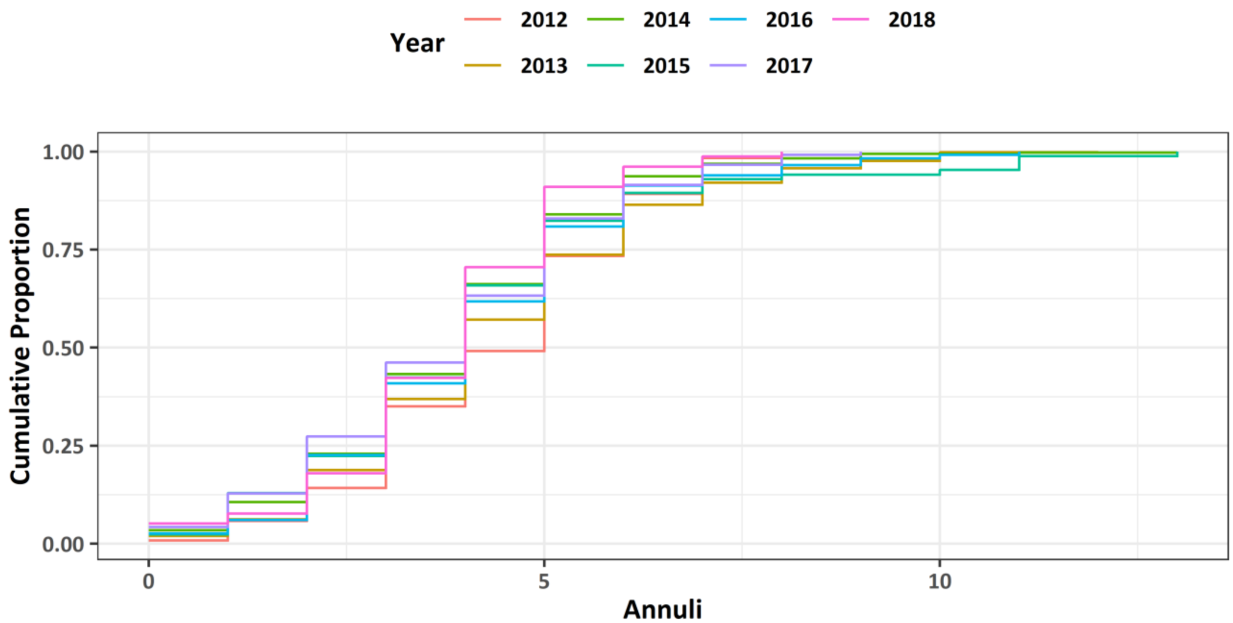

Figure 19: Empirical cumulative distribution function showing the annuli counts of American Eel retained for detailed life history information by year. Note, only years for which >30 individual eels were aged are shown.

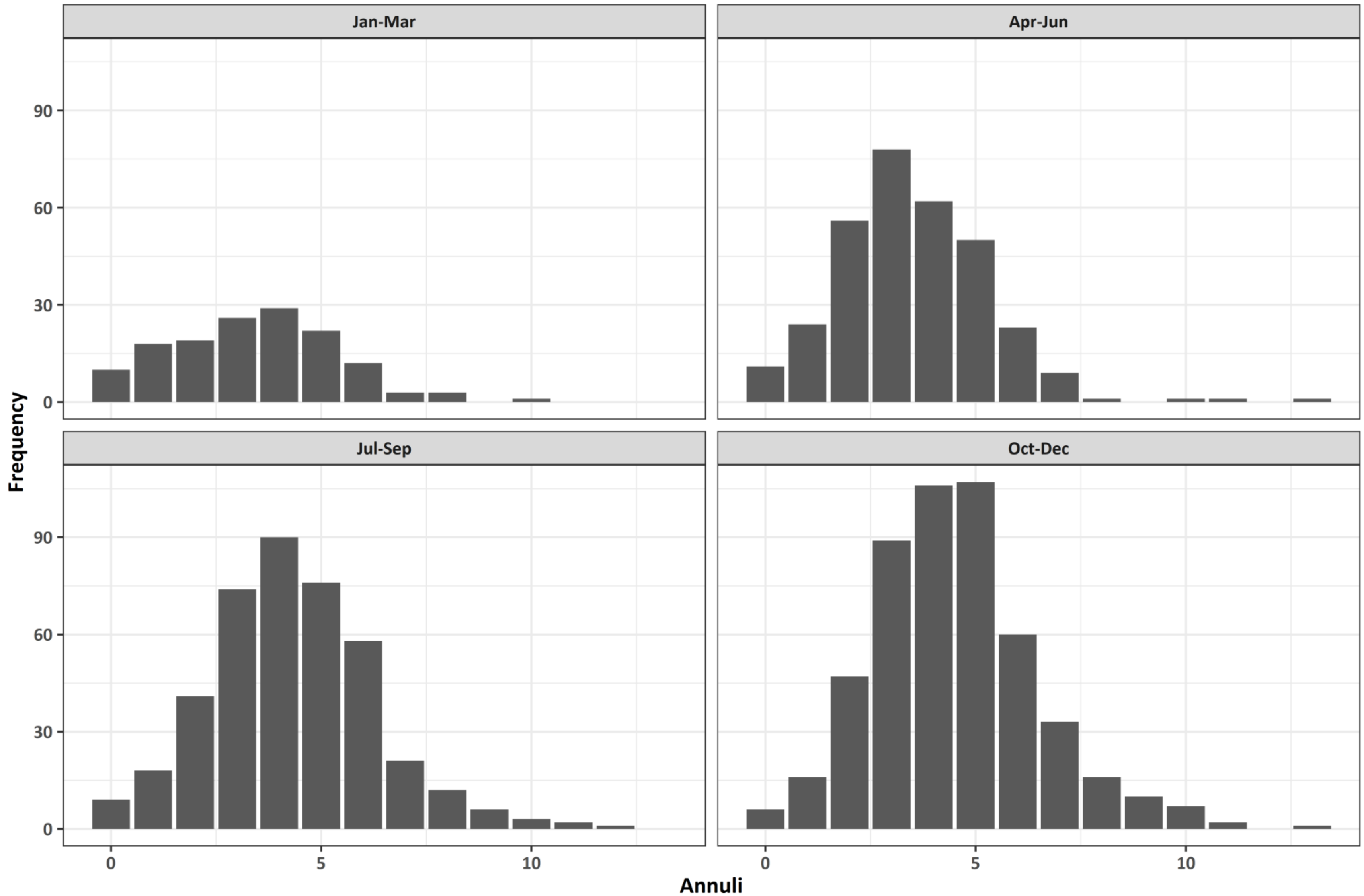

Figure 20: Annulus count composition of American Eels retained for detailed life history study by the SCDNR by year.

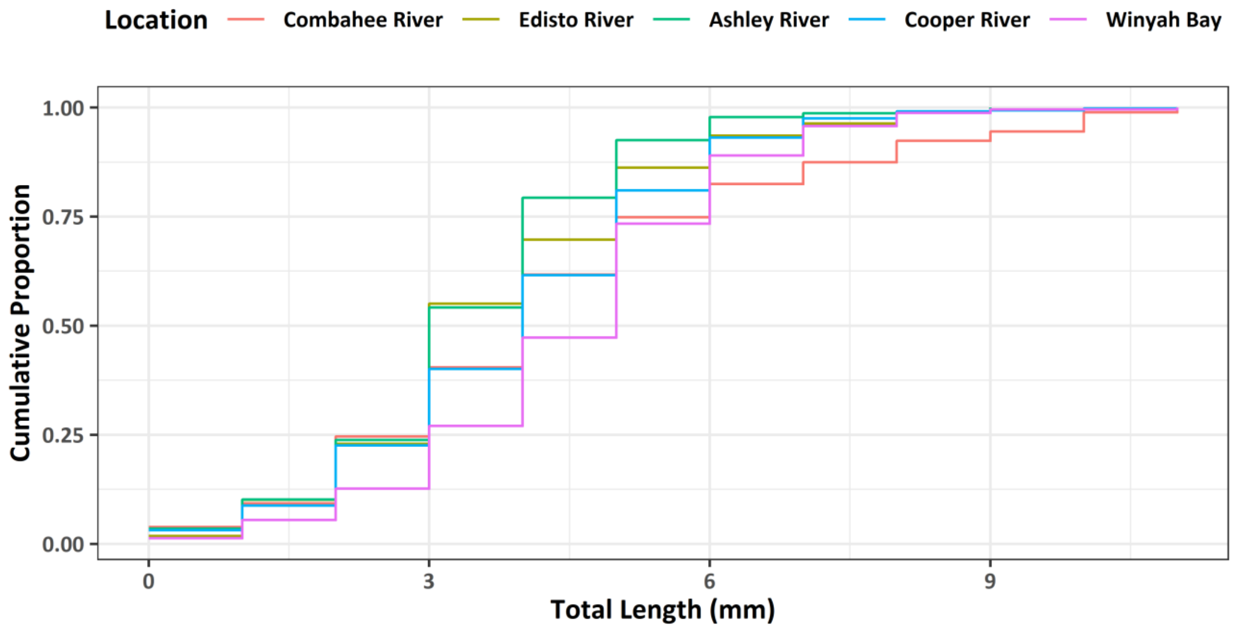

Figure 21: Empirical cumulative distribution function showing the annuli counts of American Eel retained for detailed life history information by electrofishing survey strata.

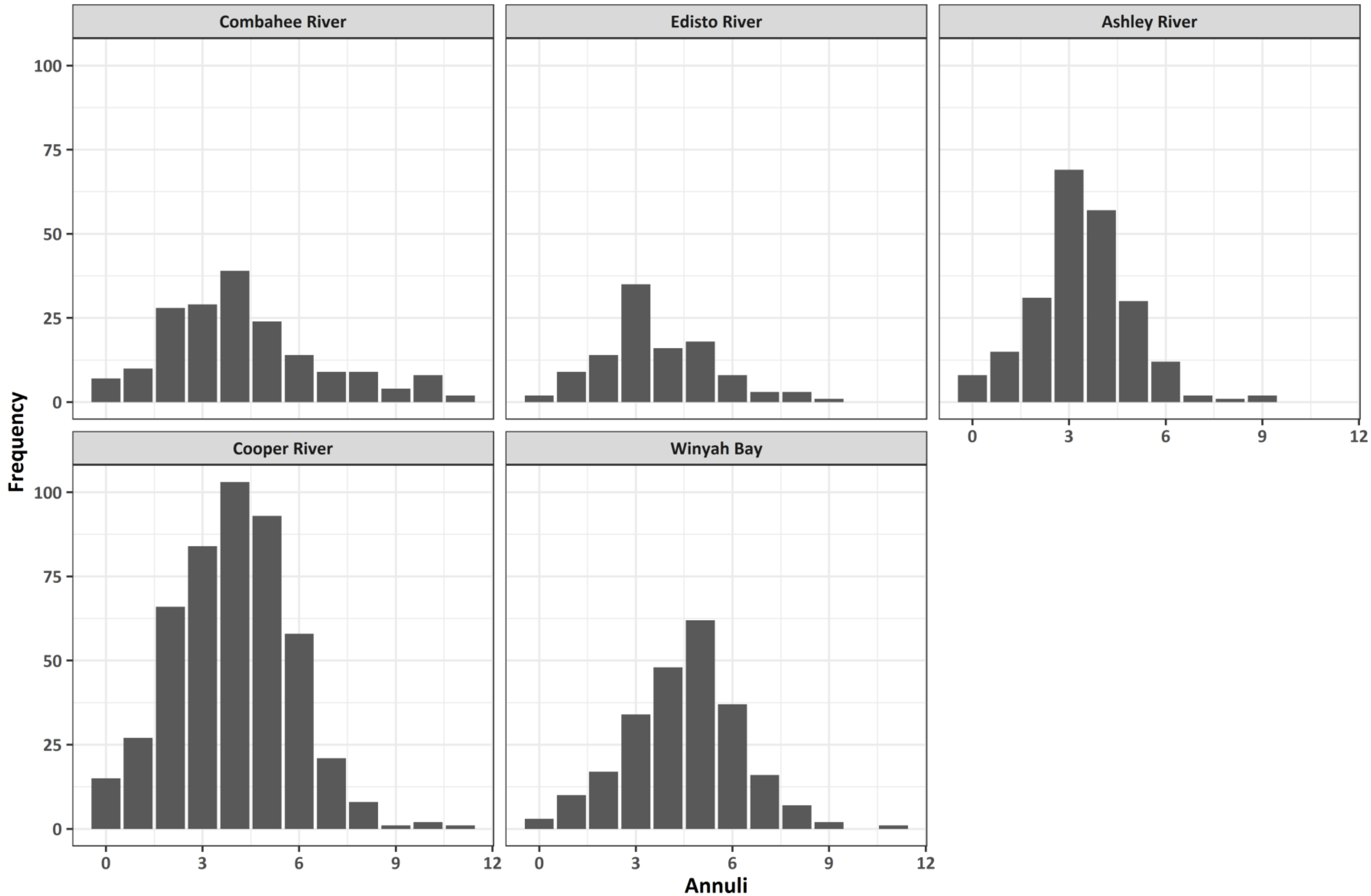

Figure 22: Annulus count composition of American Eels retained for detailed life history study by the SCDNR by electrofishing survey strata.

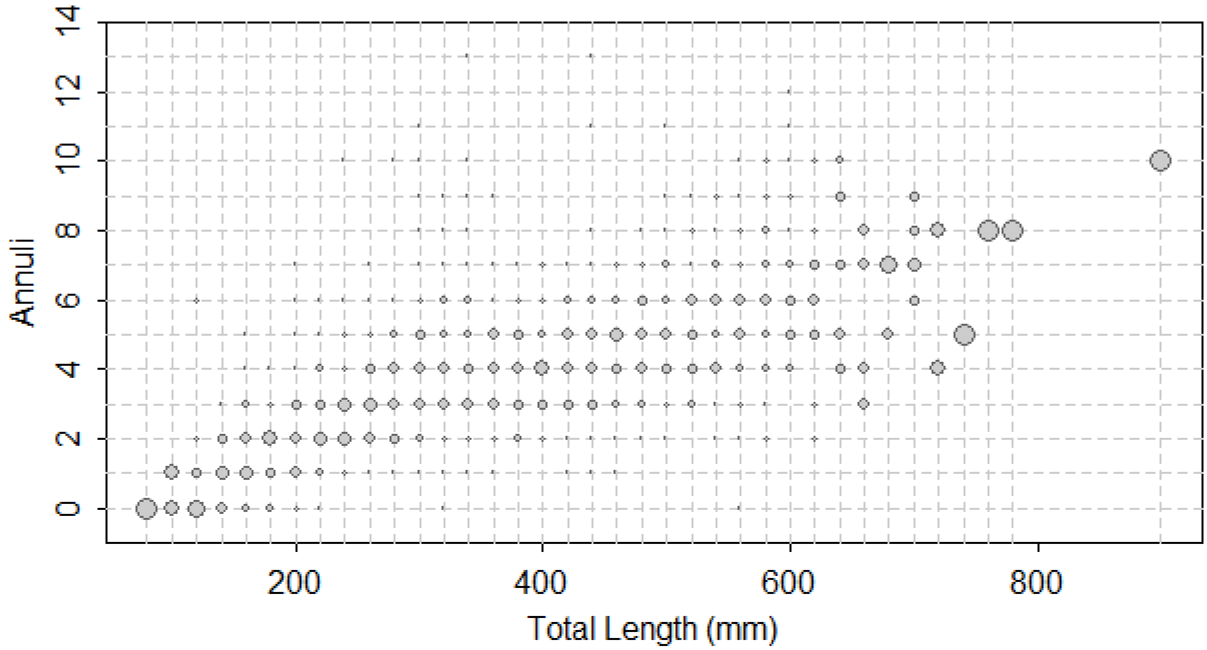

Figure 23: Raw age-length key with undifferentiated, male, and female American Eel data combined. The size of the circle represents the probabilities of size at age.

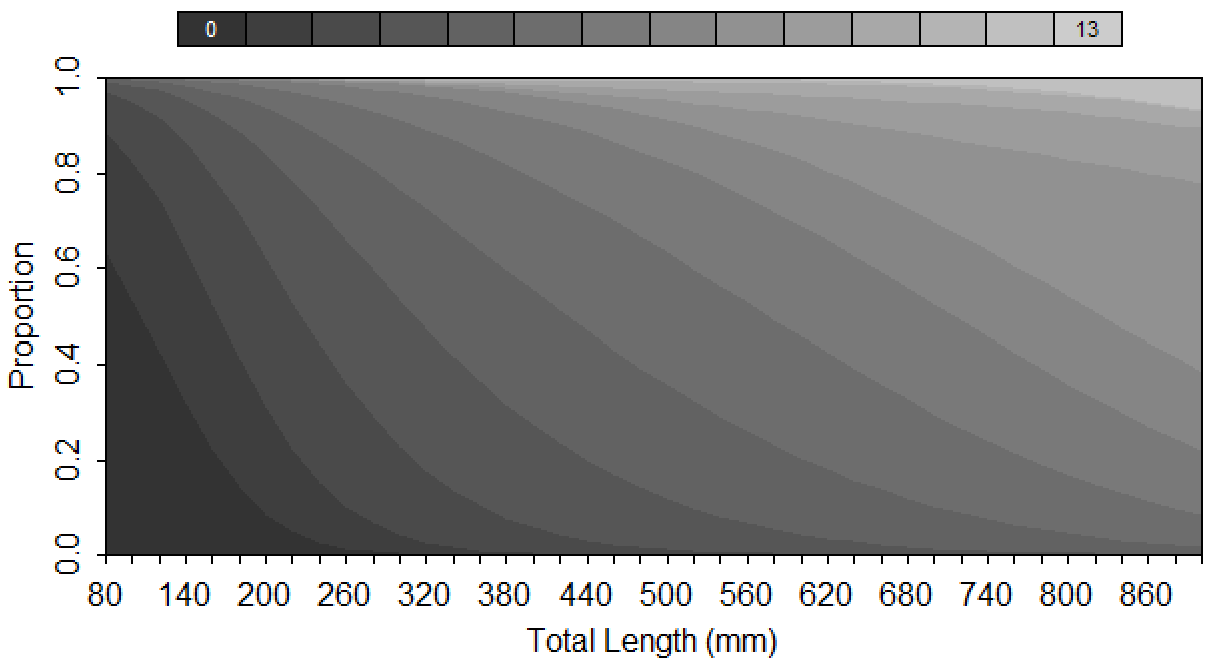

Figure 24: Smoothed age-length key with undifferentiated, male, and female American Eel data combined. The age-length key was smoothed using multinomial logistic regression.

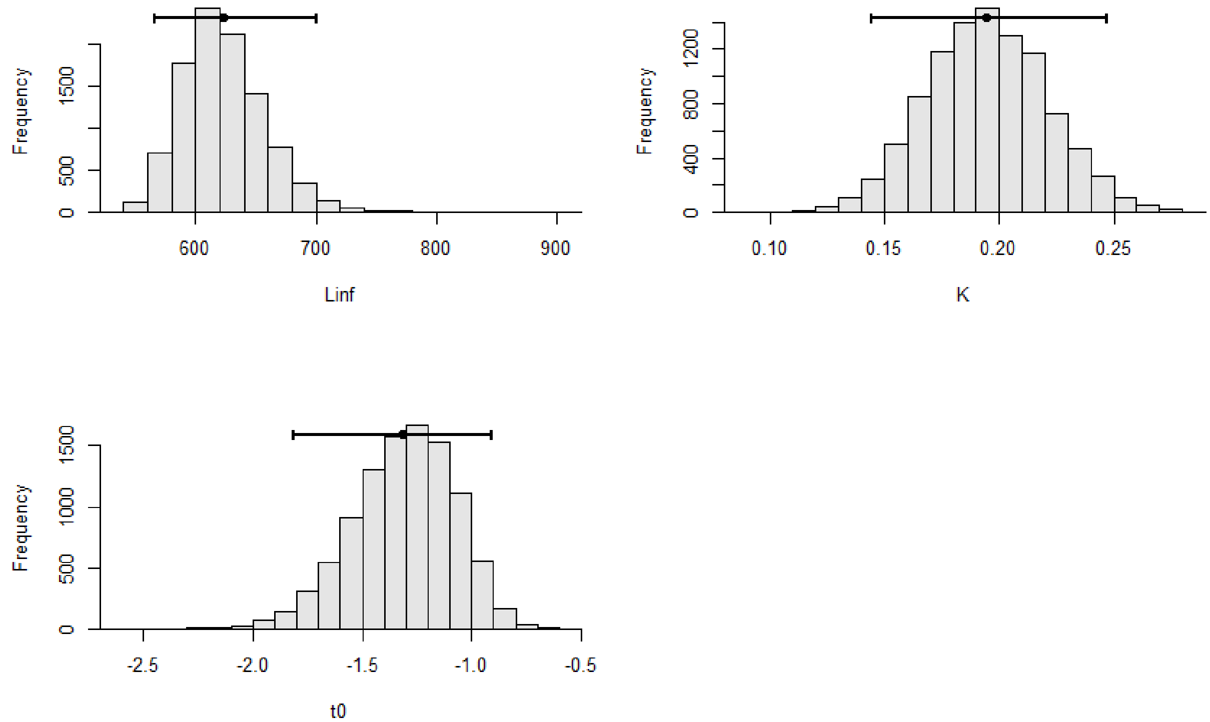

Figure 25: Parameter estimates (black dot) along with 95% CI (horizontal line) of estimate, based on 10,000 bootstraps, for the sexes pooled von Bertalanffy growth model using OTS method derived annulus counts. Also shown is a histogram of the distribution of individual bootstrap run parameter estimates.

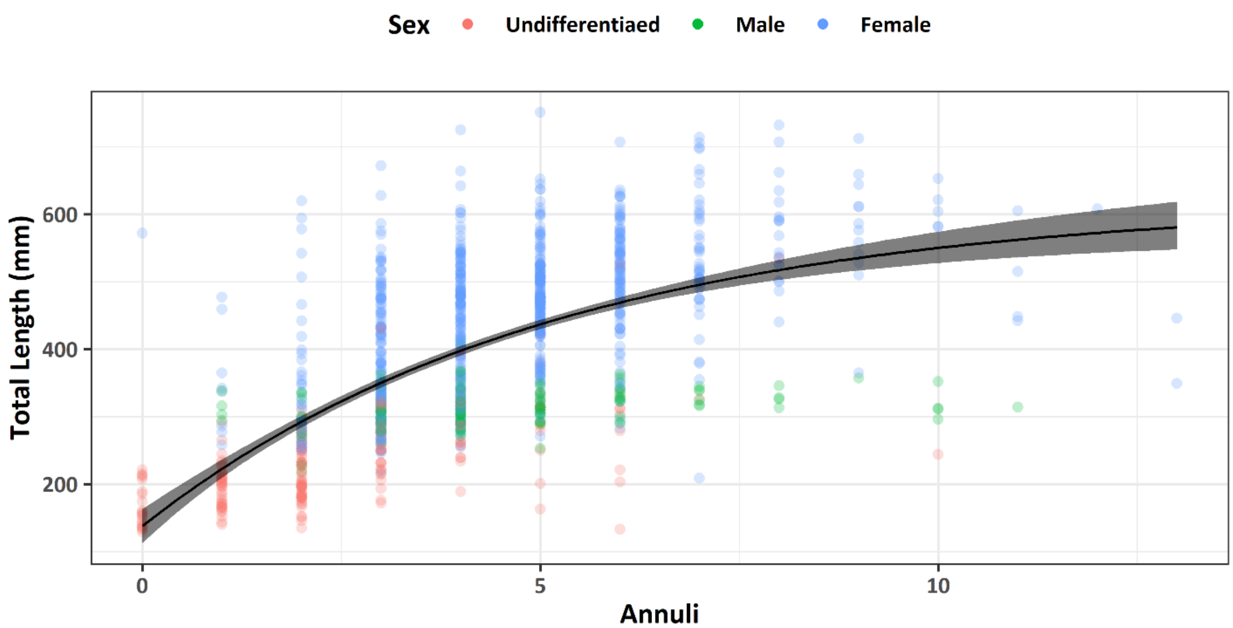

Figure 26: American Eel fitted von Bertalanffy growth curve. Curve was fit to OTS method derived annulus counts and observed total length. For this analysis, data were pooled across sex, though individual points identify undifferentiated (red circles), male (green circles), and female (blue circles) American Eels. Predicted mean size-at-age (black line) and 95% CI of mean size-at-age (gray shaded region) based on 10,000 bootstraps are shown.

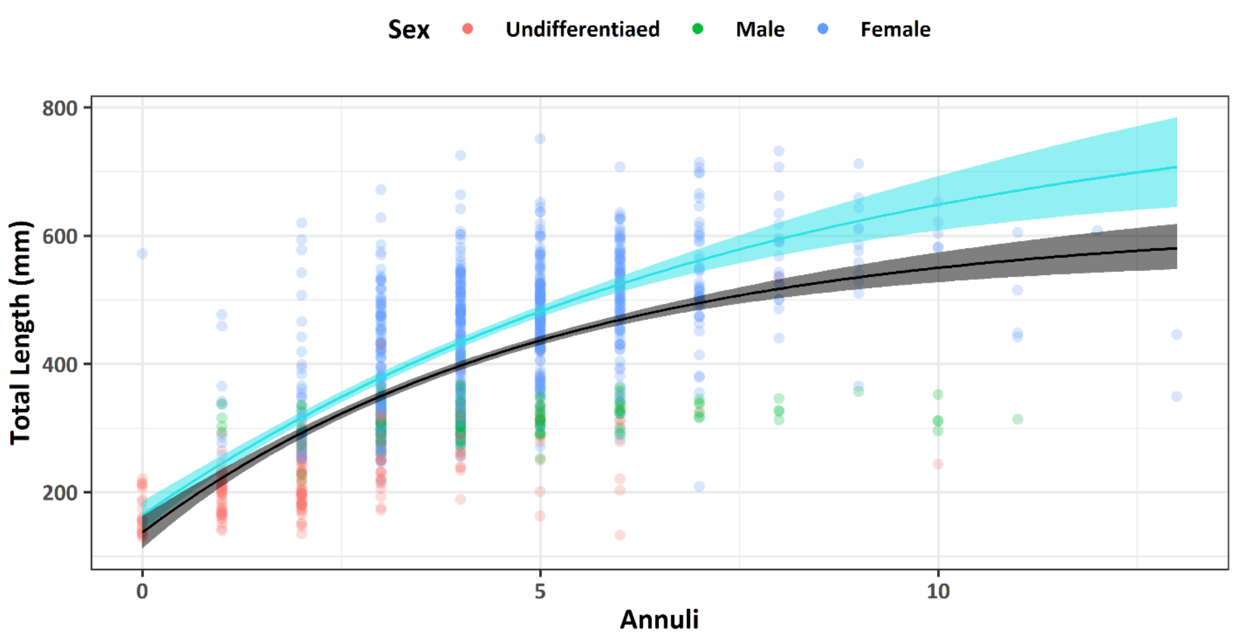

Figure 27: Same as Figure 26 but included additional von Bertalanffy predicted mean size-at-age of American Eels based on fits to WO method derived annulus counts. Predicted mean size-at-age (blue line) and 95% CI of mean size-at-age (blue shaded region) based on 10,000 bootstraps are shown.

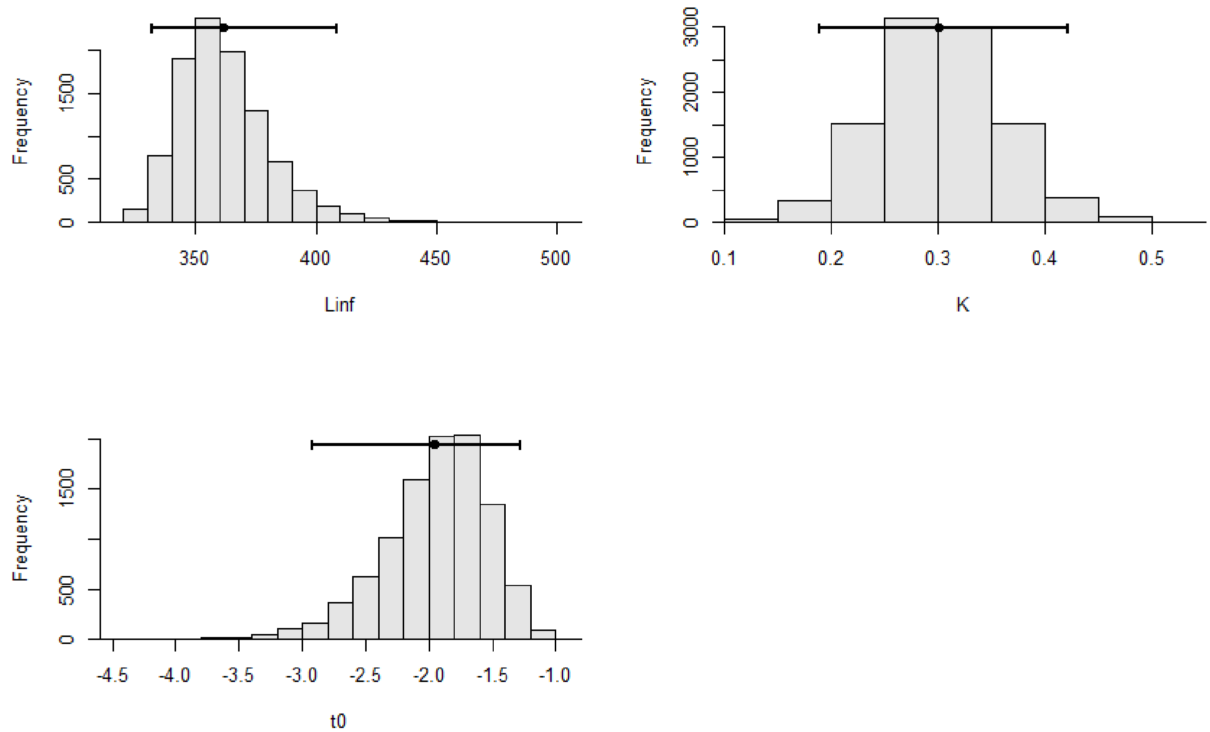

Figure 28: Parameter estimates (black dot) along with 95% CI (horizontal line) of estimate, based on 10,000 bootstraps, for the male von Bertalanffy growth model using OTS method derived annulus counts. Also shown is a histogram of the distribution of individual bootstrap run parameter estimates.

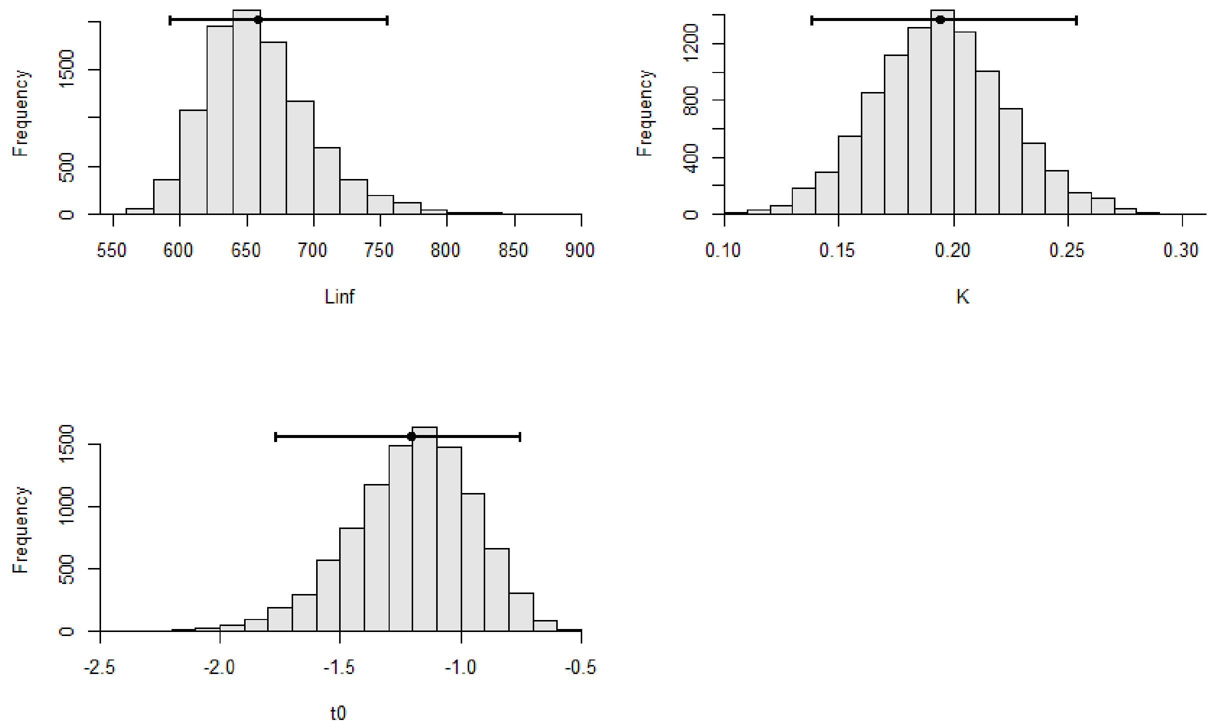

Figure 29: Parameter estimates (black dot) along with 95% CI (horizontal line) of estimate, based on 10,000 bootstraps, for the female von Bertalanffy growth model using OTS method derived annulus counts. Also shown is a histogram of the distribution of individual bootstrap run parameter estimates.

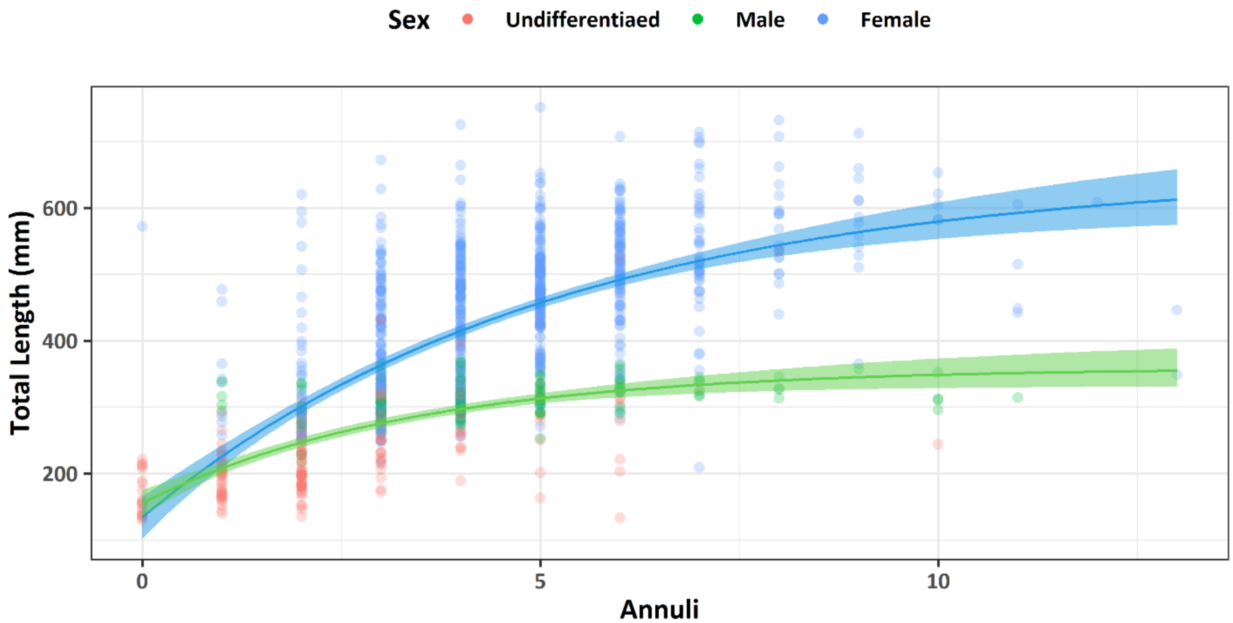

Figure 30: American Eel fitted von Bertalanffy growth curve. Curve was fit to OTS method derived annulus counts and observed total length. For this analysis, data were analyzed by sex. Shown are observed size-at-age of American Eels by sex: undifferentiated (red circles), males (green circles), and females (blue circles). Predicted mean size-at-age (lines) and 95% CI of mean size-at-age (shaded regions) are shown for males (green) and females (blue).

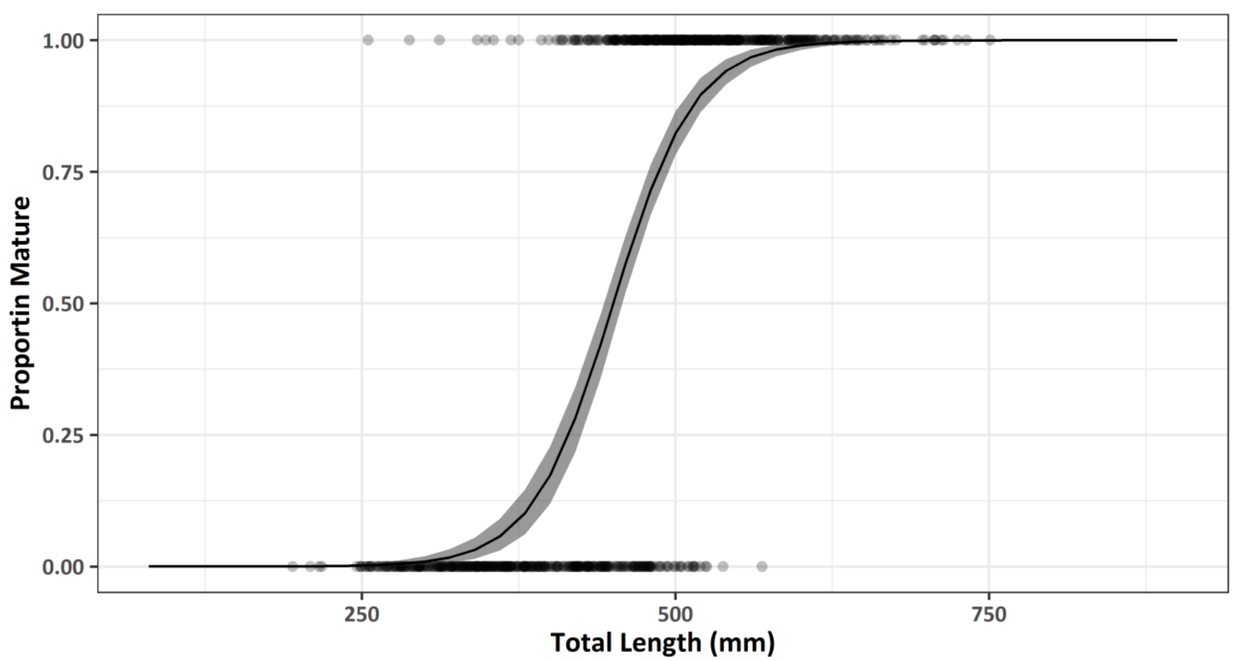

Figure 31: Size-at-maturity of female American Eels. Shown are raw maturity (dots) as a function of size along with the fitted logistic regression (black line) and 95% CI (shaded region) of the fitted curve showing the probability of being mature as a function of size.

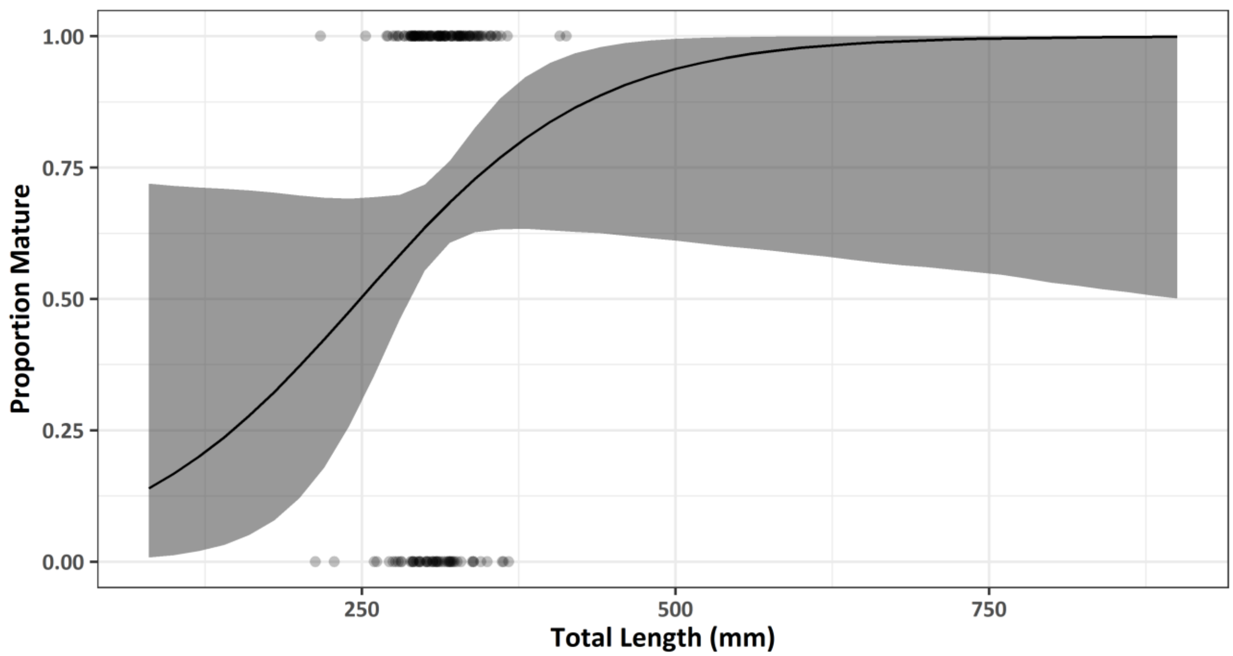

Figure 32: Size-at-maturity of female American Eels. Shown are raw maturity (dots) as a function of size along with the fitted logistic regression (black line) and 95% CI (shaded region) of the fitted curve showing the probability of being mature as a function of size. Note, though showing the results of the regression here, the regression suggested no significant relationship between size and probability of mature for male American Eels.

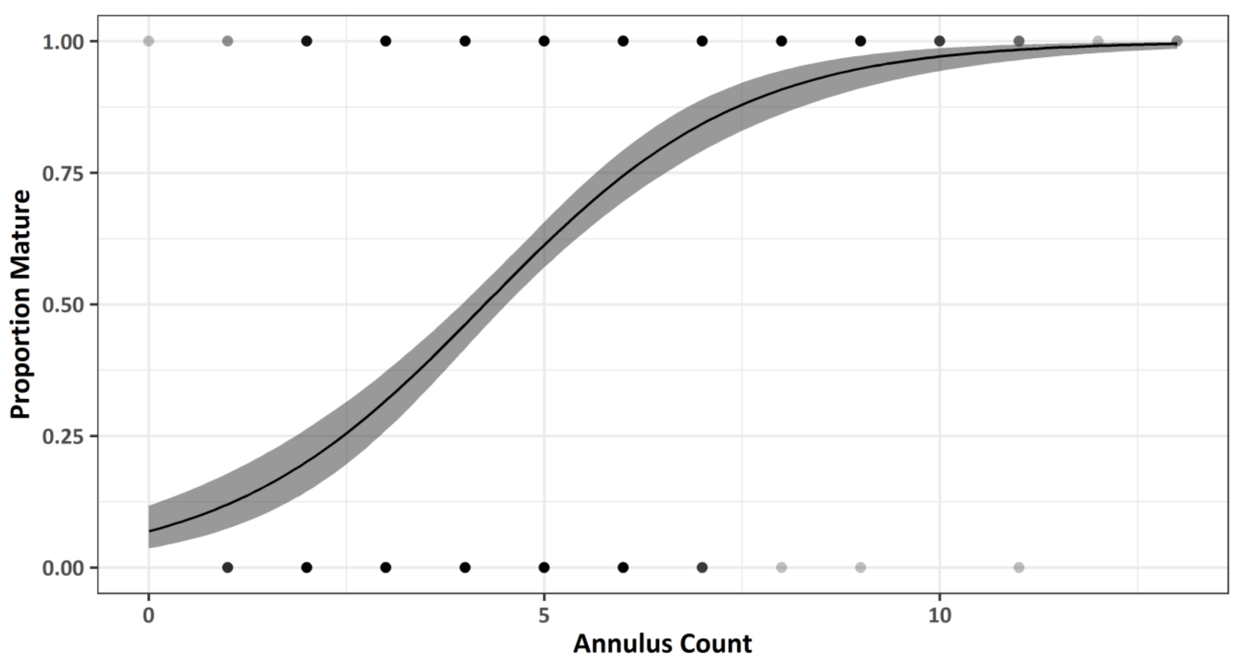

Figure 33: Age-at-maturity of male American Eels. Shown are raw maturity (dots) as a function of annulus count along with the fitted logistic regression (black line) and 95% CI (shaded region) of the fitted curve showing the probability of being mature as a function of annulus count.

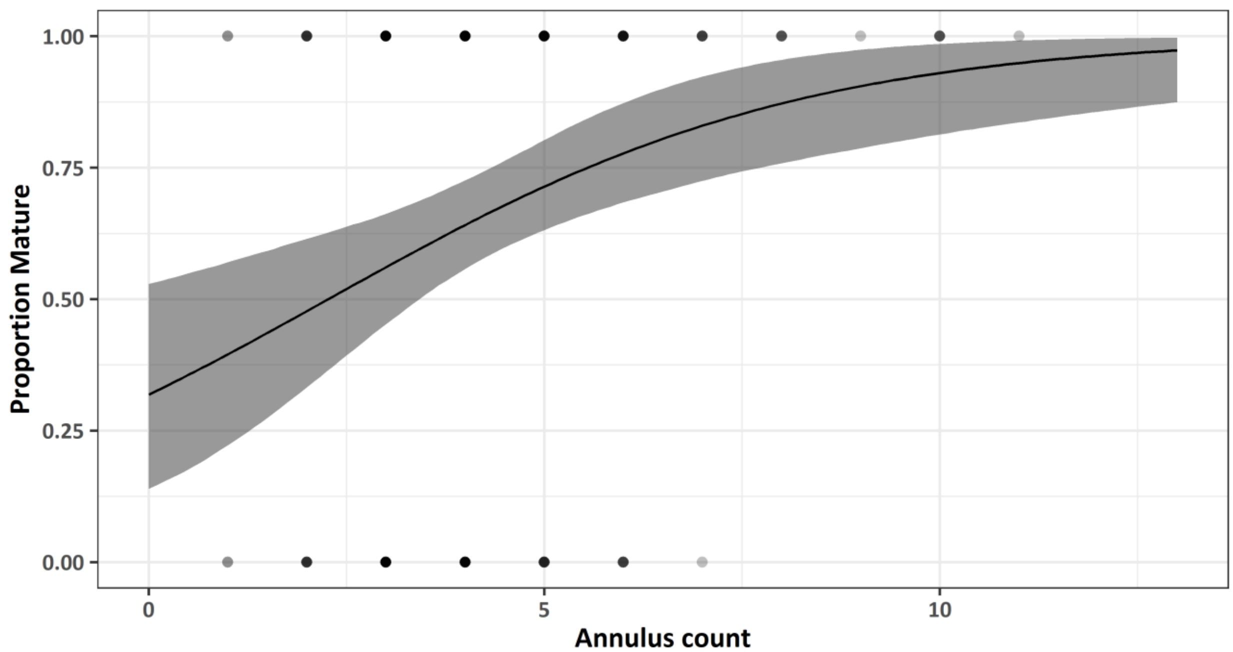

Figure 34: Age-at-maturity of male American Eels. Shown are raw maturity (dots) as a function of annulus count along with the fitted logistic regression (black line) and 95% CI (shaded region) of the fitted curve showing the probability of being mature as a function of annulus count.

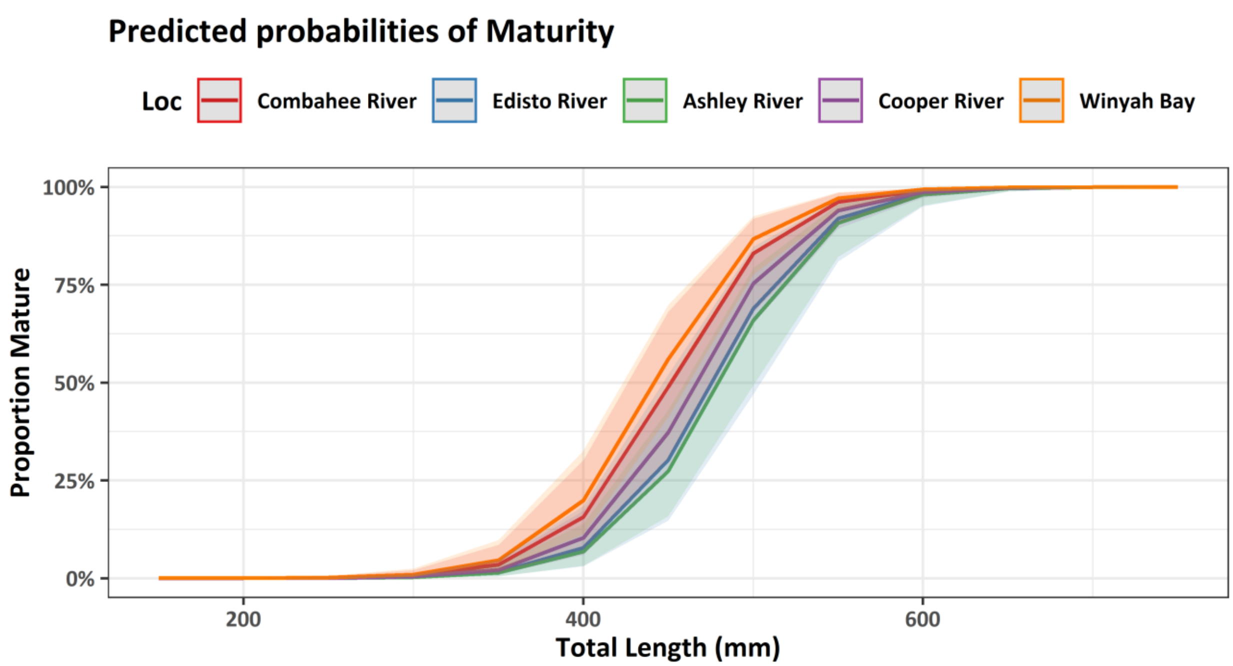

Figure 35: Variation in female American Eel probability of being mature as a function of total length (in mm) and river system. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for each river system (different colors).

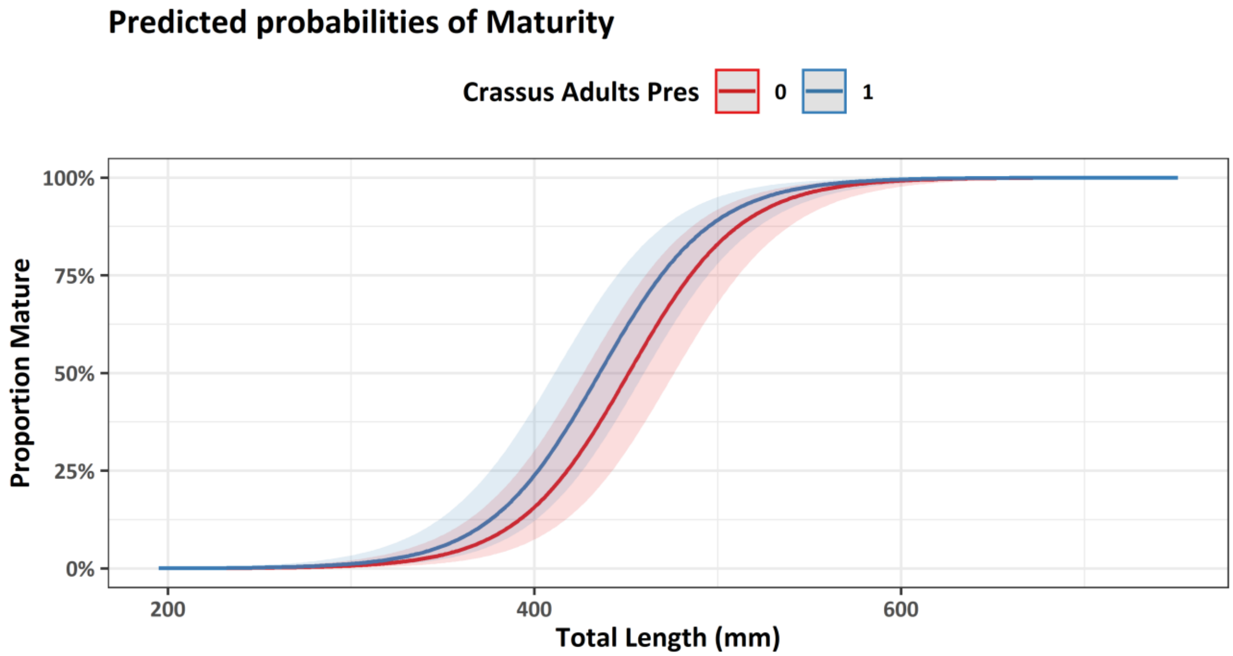

Figure 36: Variation in female American Eel probability of being mature as a function of total length (in mm) and presence of *A. crassus* adults in the swim bladder. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for swim bladders not possessing (red) and possessing (blue) adult *A. crassus*.

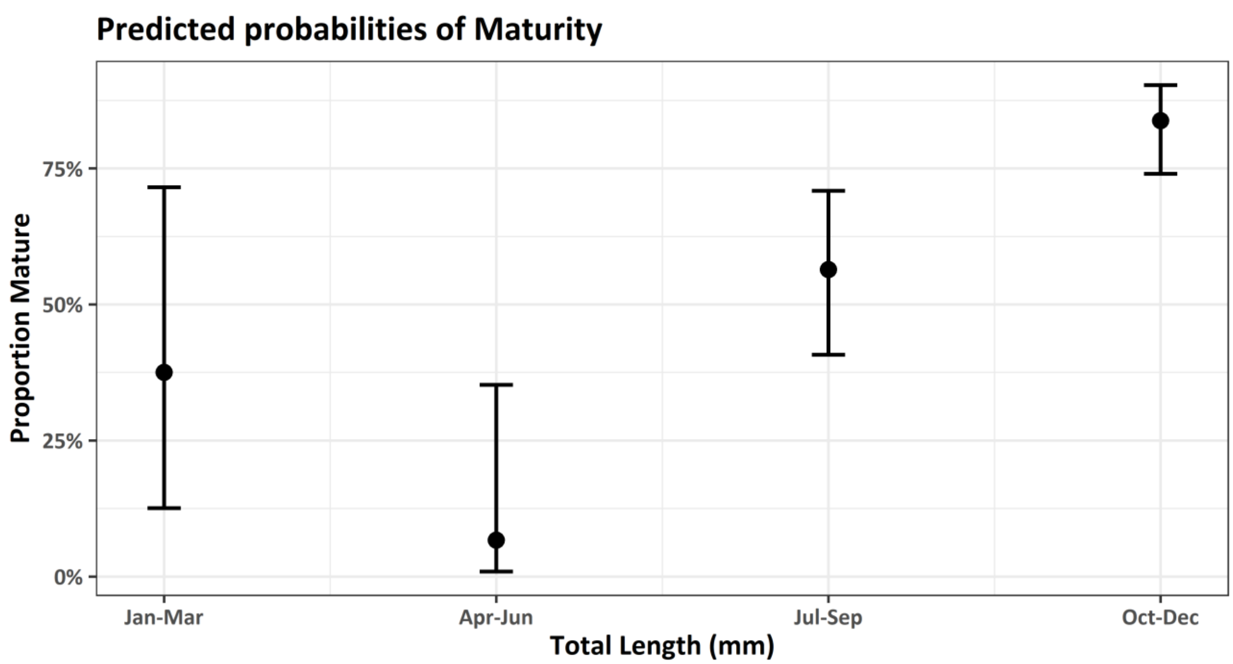

Figure 37: Variation in male American Eel probability of being mature as a function of season. Shown are the best estimate and (points) along with 95% confidence intervals (lines) for each quarter.

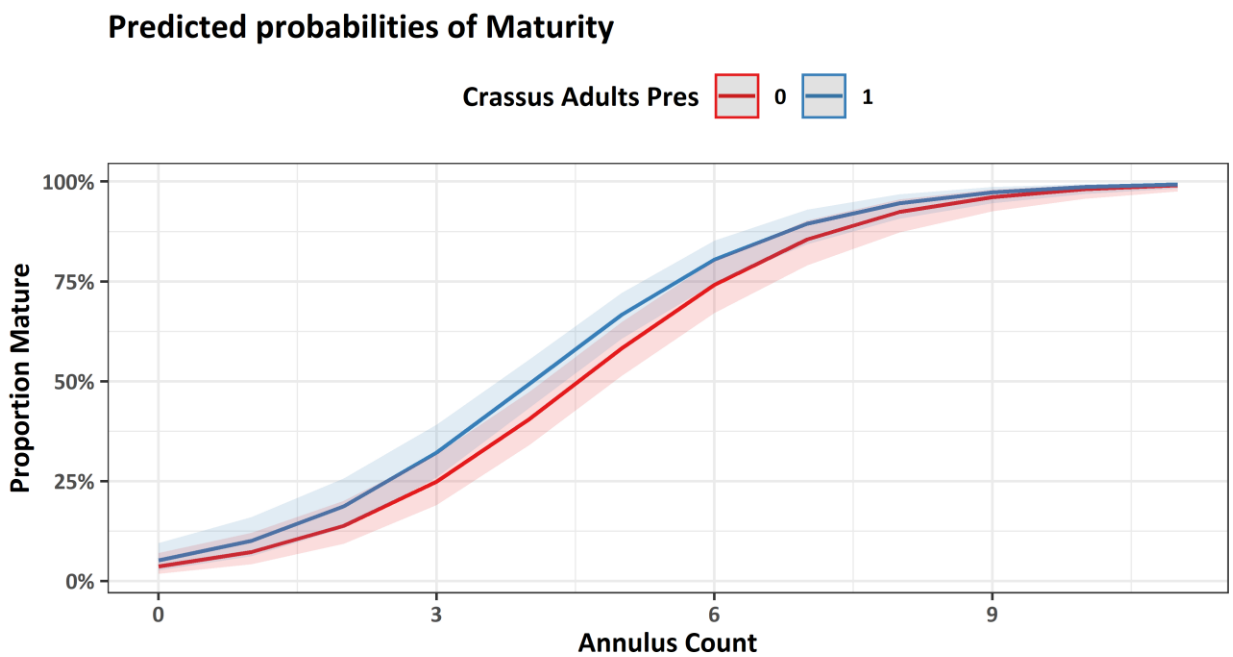

Figure 38: Variation in female American Eel probability of being mature as a function of annulus count and presence of *A. crassus* adults in the swim bladder. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for swim bladders not possessing (red) and possessing (blue) adult *A. crassus*.

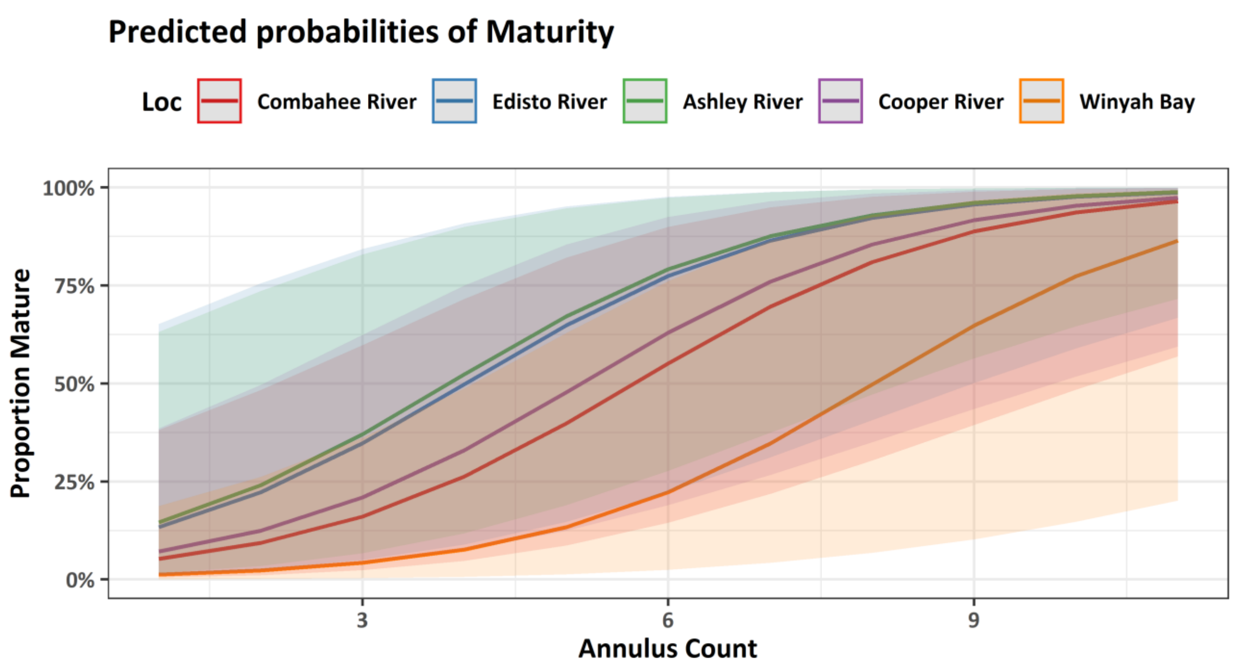

Figure 39: Variation in male American Eel probability of being mature as a function of annulus count and river system. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for each river system (different colors).

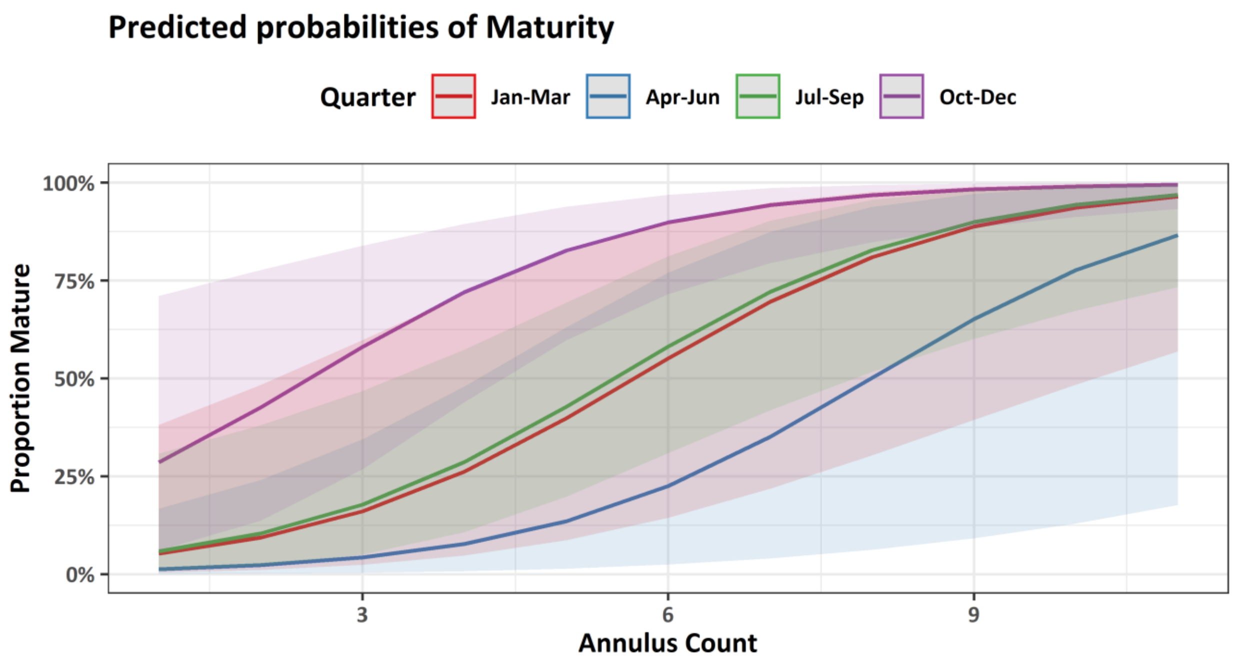

Figure 40: Variation in male American Eel probability of being mature as a function of annulus count and season. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for each river system (different colors).

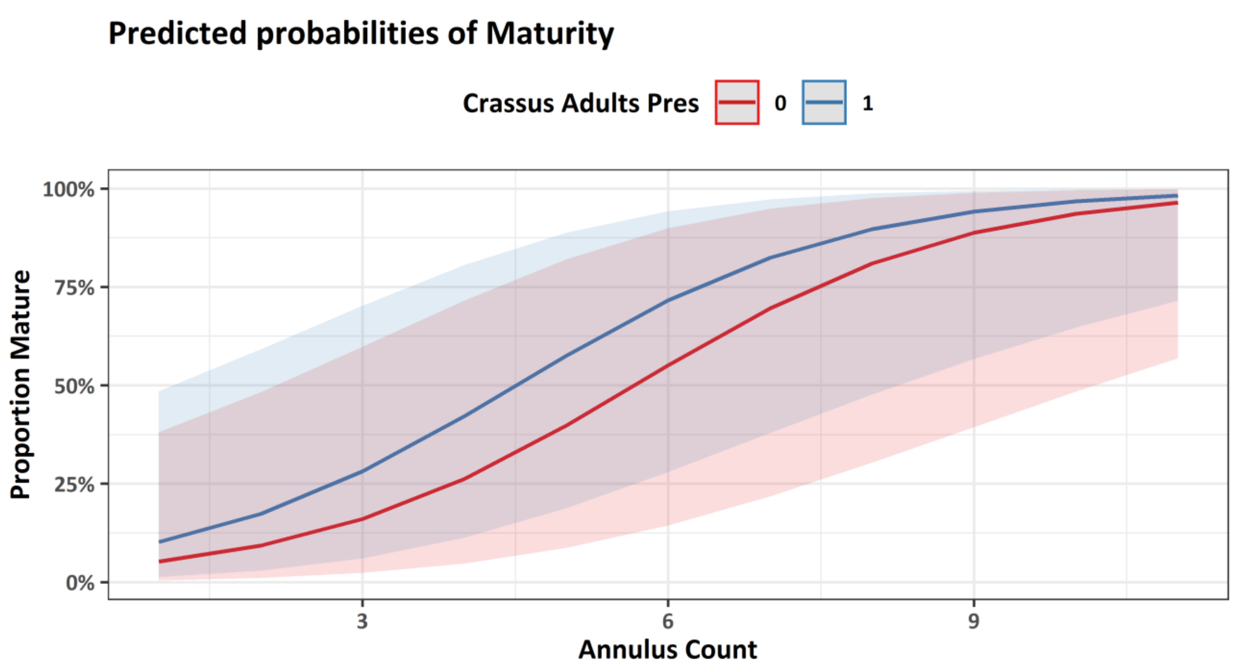

Figure 41: Variation in male American Eel probability of being mature as a function of annulus count and presence of *A. crassus* adults in the swim bladder. Shown are the fitted curves (solid lines) along with 95% confidence intervals (shaded regions) for swim bladders not possessing (red) and possessing (blue) adult *A. crassus*.